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In re Application of:

Jenkins, Peter James et al.

Application No.: 10/776,188

Filed: February 12, 2004

For: PREPARATION AND DIABETIC  
USE OF GIBBERELLINS

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Group Art Unit: 1623

Examiner: E. Peselev

Confirmation No.: 3089

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**DECLARATION UNDER 37 C.F.R. § 1.132 OF DR. PETER JENKINS**

I, PETER JAMES JENKINS, do hereby declare and state as follows:

1. I am an inventor of the subject matter described in U.S. Application No. 10/776,188 ("the '188 application"), filed February 12, 2004.
2. I have been employed by Australian Biomedical Company Pty, Ltd., from December 1995 to the present. I am a Director of this company and a member of their Scientific Research Committee and I have held these

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positions since 1995. My primary responsibilities in my present position include those of coordinating research and development.

3. My qualifications and professional training are as follows:

1972 – M.B., B.S. (Melbourne)

1979 – Fellow of the Royal Australasian College of Physicians

1973 – Intern, St. Vincent's Hospital, Melbourne

1974 – Junior Resident Medical officer, St. Vincent's Hospital, Melbourne

1975-1977 – Medical Registrar, Alfred Hospital, Melbourne

1978-1980 – Senior Registrar and Honorary Lecturer, King's College Hospital, London

1980- Present – Consultant Physician, Member of Professorial Medical Unit, Alfred Hospital, Melbourne

1996-1998 – Chairman Senior Medical Staff, Alfred Hospital Melbourne.

4. My general work experience includes the management of patients with Type I

and Type II diabetes mellitus, and includes my research experience involving

. *in vitro* and *in vivo* experiments including the use of Gibberellins.

5. I participated in the development of experiments described in Examples 1-6 of

the '188 application. Additionally, I have either performed, supervised or

overseen the performance of Examples 1-6.

6. I have read and understand the entire disclosure of the '188 application,

including the claims 1-38.

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7. I have been asked to comment on Example 5 of the '188 application at p. 21, line 19, to p. 23, line 1. Specifically, I have been asked to provide my opinion on whether Example 5 enables one skilled in the art, such as myself, to use Gibberellins to treat Type I and II diabetes. In my opinion, for the reasons set forth below, Example 5 enables the use of Gibberellins alone to treat Type II diabetes mellitus and its complications and associated conditions, and the use of Gibberellins and a substance, such as insulin, for treating Type I diabetes mellitus and its complications and associated conditions.
8. For purposes of this declaration, the experiments disclosed in Example 5 were performed under my direction and control.
9. Example 5 describes experiments with male Wistar rats that were induced with diabetes. Although Example 5 does not specifically disclose the type of diabetes exhibited by the rats, it was well known in the art as of the filing date of the '188 application that Type I diabetic humans and animals may produce trace amounts of endogenous insulin, although this typically ceases altogether after a few years. (See paragraph 7.1.1, "Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications", World Health Organization, Geneva 1999 ("the WHO Report").) A copy of the WHO Report is attached as Exhibit A.
10. Type II diabetic humans and animals typically have some endogenous insulin but produce an amount of insulin that is insufficient to adequately control their

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blood glucose level, either due to decreased insulin production and/or decreased sensitivity to insulin. (See *ibid.*, paragraph 7.2.)

11. Diabetes was induced in the rats of Example 5 by administering 60 mg/kg of streptozotocin (STZ). In my opinion, it was well known that injecting STZ is a standard procedure to induce diabetes in an animal. By way of one example only, see the discussion in "Increased Susceptibility to Streptozotocin Induced  $\beta$ -Cell Apoptosis and Delayed Autoimmune Diabetes in Alkylpurine-DNA-N-Glycolase-Deficient Mice", Cardinal, J.W. et al., *Molecular and Cellular Biology*, August 2001, p. 5605, American Society for Microbiology, attached as Exhibit B, or "Protein kinase C and the sub-sensitivity and sub-reactivity of the diabetic rat prostate gland to noradrenaline", Ramasamy, S. et al., *European Journal of Pharmacology* 434 (2002) 151-161, attached as Exhibit C . STZ acts upon  $\beta$ -cells in the pancreas and prevents endogenous production of insulin. (See *Cardinal* and *Ramasamy*.)
12. According to Example 5, a glucose sample was taken from each rat, and rats that showed a blood glucose level  $\geq$ 16 mM were considered to be unequivocally diabetic for the purposes of the experiment. A blood glucose level in the range of 4 mM to 6 mM is considered normal. (See *The New England Journal of Medicine*, SI Unit Conversion Guide, Laposata, M. (ed.) 1992 NEJM Books, Boston, pp. 82-83, attached as Exhibit D, and *Harpers Biochemistry* 24<sup>th</sup> ed., Murray, R.K., et. al. 1996, Appleton & Lange, Stamford, Connecticut, pp. 830-832, attached as Exhibit E.)

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13. In my opinion, one of ordinary skill in the art would have expected that the amount of STZ administered in Example 5 (60 mg/kg) to an animal having the body weight of a male Wistar rat (290g-330g), would result in β-cell death, thereby inducing Type I diabetes in the animal, e.g., a rat producing trace or no amounts of endogenous insulin. (See ¶ 9, *supra*.)
14. Example 5 further describes a control group of male Wistar rats injected with citrate buffer by a single tail vein injection.
15. Achieving a blood glucose level in the range of 4 mM to 6 mM, which is considered normal, in a diabetic rat or human indicates effective control of blood glucose level and effective treatment and management of complications and associated conditions of diabetes that arise from elevated blood glucose levels, e.g., ≥16 mM.
16. In Example 5, each of the five rat groups is administered with an insulin preparation as listed in the specification at p. 22, line 27 to p. 23, line 1. Each of the groups 3 to 5 is also administered with a novel anti-diabetic agent comprising Gibberellin, again as listed in the specification. These preparations were administered daily and blood glucose readings were taken every 3 days at the times indicated for a total of 84 days. Each preparation included slow-acting Lente Monotard insulin in dosage amounts measured as "units."

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17. One of ordinary skill in the art would have known that a "unit" is an international standard measurement for drug activity. It is well known that with respect to insulin dosage, a unit corresponds to the activity contained in one twenty-second (1/22) of a milligram of the pure crystalline product now adopted as the standard. (See definition of "International unit" and "international insulin unit" in *Dorland's Illustrated Medical Dictionary, 25<sup>th</sup> Edition*, 1974 W.B. Saunders Company, Philadelphia, attached as Exhibit F).
18. Blood glucose readings were obtained 2 hours and/or 5 hours after the administration and body weight change was recorded on day 30.
19. In Example 5, the preparation administered to Group No. 1 rats contained only insulin at a dosage of 4 units/rat. This preparation effectively brought the glucose level of rats identified as diabetic to a range of 4-5 mM (2 hours after injection) and 3-5 mM (5 hours after injection). These blood glucose ranges overlap with the 4 mM to 6 mM range. And, in my opinion, one of ordinary skill in the art would have considered the Group No. 1 rats to have achieved a normal glucose level.
20. In Example 5, Group No. 2 rats were administered a preparation containing insulin at a dosage of 2 units/rat subcutaneously. The Group No. 2 rats exhibited a blood glucose level ranging from 15-18 mM (2 hours after injection) and 14-16 mM (5 hours after injection). In my opinion, these blood glucose ranges are considered to be diabetic, as the range is greater than the

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normal range of 4 mM to 6 mM and overlaps with the  $\geq 16$  mM range indicative of diabetes.

21. Further, it is my opinion that the Group No. 2 rats illustrate a Type II diabetes model. Although the Group No. 2 rats were administered insulin, the amount administered was insufficient to normalize blood glucose levels to the 4-6 mM range. Because the Group No. 2 rats had a blood glucose level range considered to be diabetic and were known to have insulin, albeit in an insufficient amount to normalize blood glucose levels, the Group No. 2 rats were effectively functional as a Type II diabetes model. (See ¶ 10, *supra*.)
22. Example 5 further describes administering to each of Group Nos. 3-5 rats a preparation containing insulin at a dosage of 2 units/rat and 5 mg/kg of Gibberellin A<sub>3</sub>. The administration of insulin to Group Nos. 3-5 rats was performed subcutaneously, whilst the administration of Gibberellin A<sub>3</sub> was performed subcutaneously, intraperitoneally, and orally, respectively.
23. In Example 5, the administration of the preparation containing insulin at a dosage of 2 units/rat and Gibberellin A<sub>3</sub> (5 mg/kg) to rats in Group Nos. 3-5 effectively brought the blood glucose level of rats identified as diabetic to a range of 4-6 mM (2 hours after injection, Group No. 3; 5 hours after injection, Group Nos. 4 and 5) or 4-5 mM (2 hours after injection, Group No. 4). These blood glucose ranges are considered to be normal, as indicated on p. 5, line

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33, to p. 6, line 2, of the '188 application. Thus, the preparations administered to Group Nos. 3-5 treated diabetes.

24. It is my opinion that the treatment of Group Nos. 3-5 illustrates treating both Type I and Type II diabetes, as explained further below.

25. A Type I diabetic animal produces trace amounts or no amount of endogenous insulin. (See ¶ 9, *supra*.) As discussed in ¶ 13, *supra*, the Group Nos. 1-5 rats prior to administration of the preparation were all Type I diabetic animals, i.e., all Group Nos. 1-5 rats initially had Type I diabetes prior to administration of the preparation.

26. A standard treatment of Type I diabetes usually requires administration of insulin. (See *Martindale: the complete drug reference 33<sup>rd</sup> Edition*; Sweetman, S.C. (ed.) 2002 Pharmaceutical Press, London, attached as Exhibit G.) In the standard treatment, the amount of insulin must be carefully monitored to avoid potential problems with producing hypoglycemia.

27. The experiments with Group No. 2 rats show that administration of 2 units/rat of insulin is insufficient to normalize blood glucose levels. (See ¶ 21, *supra*.) In contrast, the experiments with Group No. 1 rats show that a dosage of 4 units/rat of insulin normalized blood glucose levels.

28. However, the preparations administered to Group Nos. 3-5 rats containing an insufficient amount of insulin (2 units/rat) and a novel anti-diabetic agent, Gibberellin A<sub>3</sub>, resulted in rats having a normal blood glucose range. Thus, it

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is my opinion that Example 5 fully enables effective treatment of Type I diabetes by the administration of Gibberellin A<sub>3</sub> in combination with a substance such as insulin. Because the dosage of insulin in Groups 3-5 is less than that required to normalize blood glucose levels, the potential problems with producing hypoglycemia due to excess insulin are also avoided.

29. Further, it is my opinion that the treatment of Group Nos. 3-5 rats also clearly illustrates the treatment of Type II diabetes. Upon administering the preparation containing a dosage of insulin of 2 units/rat, the rats of Group Nos. 3-5 effectively contained an insufficient amount of endogenous insulin to effectively control blood glucose levels as in the Type II diabetic model, just like the Group No. 2 rats. (See ¶ 21, *supra*.)
30. However, unlike the Group No. 2 rats, the Group Nos. 3-5 rats were also administered with a novel anti-diabetic agent, Gibberellin A<sub>3</sub>. Because the administration of 2 units/rat insulin replicates the situation of attaining Type II diabetes, where small amounts of insulin may exist but in an insufficient amount to control blood glucose levels, these rats were effectively treated by the administration of Gibberellin A<sub>3</sub> alone.
31. The results from Group Nos. 3-5 rats indicate that sub-cutaneous, intraperitoneal, and oral administration of Gibberellin A<sub>3</sub> are equally effective

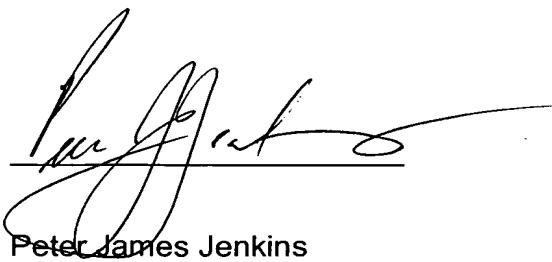
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at lowering blood glucose ranges to a normal level in Type I and II diabetic patients.

32. Example 5 illustrates how administration of Gibberellin A<sub>3</sub> alone to a male Wistar rat having insufficient insulin to control its blood glucose level, i.e., a Type II diabetic, results in attainment of a normal blood glucose range.
33. Accordingly, it is my opinion that Example 5 enables the use of Gibberellins in combination with a substance, such as insulin, in a method of treating Type I diabetes and its complications and associated conditions.
34. Further, it is also my opinion that Example 5 enables the use of Gibberellins alone in a method of treating Type II diabetes and its complications and associated conditions.

I further declare that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true, and understand that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. 1001), and may jeopardize the enforceability of any patent issuing from the '188 application.



Peter James Jenkins



22 August 2005

[Date]

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# **Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications**

Report of a WHO Consultation

**Part 1: Diagnosis and Classification of  
Diabetes Mellitus**



**World Health Organization  
Department of Noncommunicable Disease Surveillance  
Geneva**

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## **1. Introduction**

In the late 1970s both WHO (1) and the National Diabetes Data Group (2) produced new diagnostic criteria and a new classification system for diabetes mellitus. This brought order to a chaotic situation in which nomenclature varied and diagnostic criteria showed enormous variations using different oral glucose loads. In 1985 WHO slightly modified their criteria to coincide more closely with the NDDG values (3). There are now many data available, and also much more aetiological information has appeared. It seemed timely to re-examine the issues and to update and refine both the classification and the criteria, and to include a definition of the "Metabolic Syndrome".

An American Diabetes Association (ADA) expert group was convened to discuss these issues. It published its recommendations in 1997 (4). WHO convened a Consultation on the same subject in London, United Kingdom, in December 1996. In general, the ADA and WHO groups reached similar conclusions.

The provisional report of the WHO Consultation (5) solicited comments which were considered in preparing the present report. Both the provisional and the present report were prepared by Professor K.G.M.M. Alberti and Professor P.Z. Zimmet on behalf of the members of the Consultation. The meeting was made possible with financial support from Bayer, UK; Bayer, Germany; Novo Nordisk, Copenhagen, Denmark; and The Institute for Diabetes Discovery, New Haven, USA.

## **2. Definition and diagnostic criteria for diabetes mellitus and other categories of glucose intolerance**

### **2.1 Definition**

The term diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non-ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death. Often symptoms are not severe, or may be absent, and consequently hyperglycaemia sufficient to cause pathological and functional changes may be present for a long time before the diagnosis is made. The long-term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation, Charcot joints, and features of autonomic dysfunction, including sexual dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease.

Several pathogenetic processes are involved in the development of diabetes. These include processes which destroy the beta cells of the pancreas with consequent insulin deficiency, and others that result in resistance to insulin action. The abnormalities of carbohydrate, fat and protein metabolism are due to deficient action of insulin on target tissues resulting from insensitivity or lack of insulin.

## **2.2 Diagnosis and diagnostic criteria**

### **2.2.1 Diagnosis**

If a diagnosis of diabetes is made, the clinician must feel confident that the diagnosis is fully established since the consequences for the individual are considerable and lifelong. The requirements for diagnostic confirmation for a person presenting with severe symptoms and gross hyperglycaemia differ from those for the asymptomatic person with blood glucose values found to be just above the diagnostic cut-off value. Severe hyperglycaemia detected under conditions of acute infective, traumatic, circulatory or other stress may be transitory and should not in itself be regarded as diagnostic of diabetes. The diagnosis of diabetes in an asymptomatic subject should *never* be made on the basis of a single abnormal blood glucose value. For the asymptomatic person, at least one additional plasma/blood glucose test result with a value in the diabetic range is essential, either fasting, from a random (casual) sample, or from the oral glucose tolerance test (OGTT). If such samples fail to confirm the diagnosis of diabetes mellitus, it will usually be advisable to maintain surveillance with periodic re-testing until the diagnostic situation becomes clear. In these circumstances, the clinician

should take into consideration such additional factors as ethnicity, family history, age, adiposity, and concomitant disorders, before deciding on a diagnostic or therapeutic course of action. An alternative to blood glucose estimation or the OGTT has long been sought to simplify the diagnosis of diabetes. Glycated haemoglobin, reflecting average glycaemia over a period of weeks, was thought to provide such a test. Although in certain cases it gives equal or almost equal sensitivity and specificity to glucose measurement (6), it is not available in many parts of the world and is not well enough standardized for its use to be recommended at this time.

### ***2.2.2 Diabetes in children***

Diabetes in children usually presents with severe symptoms, very high blood glucose levels, marked glycosuria, and ketonuria. In most children the diagnosis is confirmed without delay by blood glucose measurements, and treatment (including insulin injection) is initiated immediately, often as a life-saving measure. An OGTT is neither necessary nor appropriate for diagnosis in such circumstances. A small proportion of children and adolescents, however, present with less severe symptoms and may require fasting blood glucose measurement and/or an OGTT for diagnosis.

### **2.3 Diagnostic criteria**

The clinical diagnosis of diabetes is often prompted by symptoms such as increased thirst and urine volume, recurrent infections, unexplained weight loss and, in severe cases, drowsiness and coma; high levels of glycosuria are usually present. A single blood glucose estimation in excess of the

diagnostic values indicated in Figure 1 (black zone) establishes the diagnosis in such cases. Figure 1 also defines levels of blood glucose below which a diagnosis of diabetes is unlikely in non-pregnant individuals. These criteria are as in the 1985 report (3). For clinical purposes, an OGTT to establish diagnostic status need only be considered if casual blood glucose values lie in the uncertain range (i.e. between the levels that establish or exclude diabetes) and fasting blood glucose levels are below those which establish the diagnosis of diabetes. If an OGTT is performed, it is sufficient to measure the blood glucose values while fasting and at 2 hours after a 75 g oral glucose load (Annexes 1 and 2). For children the oral glucose load is related to body weight: 1.75 g per kg. The diagnostic criteria in children are the same as for adults. Diagnostic interpretations of the fasting and 2-h post-load concentrations in non-pregnant subjects are shown in Table 1.

### *2.3.1 Change in diagnostic value for fasting plasma/blood glucose concentrations*

The major change recommended in the diagnostic criteria for diabetes mellitus is the lowering of the diagnostic value of the fasting plasma glucose concentration to  $7.0 \text{ mmol l}^{-1}$  ( $126 \text{ mg dl}^{-1}$ ) and above (3), from the former level of  $7.8 \text{ mmol l}^{-1}$  ( $140 \text{ mg dl}^{-1}$ ) and above. For whole blood the proposed new level is  $6.1 \text{ mmol l}^{-1}$  ( $110 \text{ mg dl}^{-1}$ ) and above, from the former  $6.7 \text{ mmol l}^{-1}$  ( $120 \text{ mg dl}^{-1}$ ).

The new fasting criterion is chosen to represent a value which is at the upper end of the range that corresponds in diagnostic significance in many persons to that of the 2-h post-load concentration, which is not changed. This equivalence has

been established from several population-based studies (6–8) and it also represents an optimal cut-off point to separate the components of bimodal frequency distributions of fasting plasma glucose concentrations seen in several populations. Furthermore, several studies have shown increased risk of microvascular disease in persons with fasting plasma glucose concentrations of  $7.0 \text{ mmol l}^{-1}$  ( $126 \text{ mg dl}^{-1}$ ) and over (6), and of macrovascular disease in persons with such fasting concentrations, even in those with 2-h values of  $< 7.8 \text{ mmol l}^{-1}$  ( $140 \text{ mg dl}^{-1}$ ) (9). Nevertheless, in less obese subjects, in some ethnic groups and in the elderly lower fasting glucose levels may be seen in persons who have 2-h post-load glucose values that are diagnostic for diabetes.

### ***2.3.2 Epidemiological studies***

For population studies of glucose intolerance and diabetes, individuals have been classified by their blood glucose concentration measured after an overnight fast and/or 2 h after a 75 g oral glucose load. Since it may be difficult to be sure of the fasting state, and because of the strong correlation between fasting and 2-h values, epidemiological studies or diagnostic screening have in the past been restricted to the 2-h values only (Table 1). Whilst this remains the single best choice, if it is not possible to perform the OGTT (e.g. for logistical or economic reasons), the fasting plasma glucose alone may be used for epidemiological purposes. It has now been clearly shown, however, that some of the individuals identified by the new fasting values differ from those identified by 2-h post glucose challenge values (10,11). The latter include the elderly (12) and those with less obesity, such as many Asian populations. On the other hand, middle-aged, more obese

patients are more likely to have diagnostic fasting values (10). Overall population prevalence may (13) or may not (7,10,14) be found to differ when estimates using fasting and 2-h values are compared.

### *2.3.3 Individual diagnosis*

The requirements for individual diagnosis differ from those of population studies. The diagnosis should not be based on a single glucose determination but requires confirmatory symptoms or blood/plasma determination. Diagnosis requires the identification of people at risk for development of complications in whom early preventive strategies are indicated. Ideally therefore both the 2-h and the fasting value should be used. These recommendations contrast with those of the ADA Expert Committee which gives primacy to the fasting plasma glucose (4).

### **3. Classification**

#### **3.1 Earlier classifications**

The first widely accepted classification of diabetes mellitus was published by WHO in 1980 (1) and, in modified form, in 1985 (3). The 1980 and 1985 classifications of diabetes mellitus and allied categories of glucose intolerance included clinical classes and two statistical risk classes. The 1980 Expert Committee proposed two major classes of diabetes mellitus and named them, IDDM or Type 1, and NIDDM or Type 2. In the 1985 Study Group Report the terms Type 1 and Type 2 were omitted, but the classes IDDM and NIDDM were retained, and a class of Malnutrition-related Diabetes Mellitus (MRDM) was introduced. In both the 1980 and 1985 reports other classes of diabetes included Other Types and Impaired Glucose Tolerance (IGT) as well as Gestational Diabetes Mellitus (GDM). These were reflected in the subsequent International Nomenclature of Diseases (IND) in 1991, and the tenth revision of the International Classification of Diseases (ICD-10) in 1992. The 1985 classification was widely accepted and is used internationally. It represented a compromise between clinical and aetiological classification and allowed classification of individual subjects and patients in a clinically useful manner even when the specific cause or aetiology was unknown. The recommended classification includes both staging of diabetes mellitus based on clinical descriptive criteria and a complementary aetiological classification.

### **3.2 Revised classification**

The classification encompasses both *clinical stages* and *aetiological types* of diabetes mellitus and other categories of hyperglycaemia, as suggested by Kuzuya and Matsuda (15).

The clinical staging reflects that diabetes, regardless of its aetiology, progresses through several clinical stages during its natural history. Moreover, individual subjects may move from stage to stage in either direction. Persons who have, or who are developing, diabetes mellitus can be categorized by stage according to the clinical characteristics, even in the absence of information concerning the underlying aetiology. The classification by aetiological type results from improved understanding of the causes of diabetes mellitus.

#### **3.2.1 Application of the new classification**

The new classification contains stages which reflect the various degrees of hyperglycaemia in individual subjects with any of the disease processes which may lead to diabetes mellitus.

All subjects with diabetes mellitus can be categorized according to clinical stage, and this is achievable in all circumstances. The stage of glycaemia may change over time depending on the extent of the underlying disease processes (Figure 2). The disease process may be present but may not have progressed far enough to cause hyperglycaemia. The aetiological classification reflects the fact that the defect or

process which may lead to diabetes may be identifiable at any stage in the development of diabetes – even at the stage of normoglycaemia. Thus the presence of islet cell antibodies in a normoglycaemic individual makes it likely that that person has the Type 1 autoimmune process. Unfortunately, there are few sensitive or highly specific indicators of the Type 2 process at present, although these are likely to be revealed as aetiology is more clearly defined. The same disease processes can cause impaired fasting glycaemia and/or impaired glucose tolerance without fulfilling the criteria for the diagnosis of diabetes mellitus. In some individuals with diabetes, adequate glycaemic control can be achieved with weight reduction, exercise and/or oral agents. These individuals, therefore, do not require insulin and may even revert to IGT or normoglycaemia. Other individuals require insulin for adequate glycaemic control but can survive without it. These individuals, by definition, have some residual insulin secretion. Individuals with extensive beta-cell destruction, and therefore no residual insulin secretion, require insulin for survival. The severity of the metabolic abnormality can either regress (e.g. with weight reduction), progress (e.g. with weight gain), or stay the same.

### **3.3 Terminology (Table 2)**

It is recommended that the terms “insulin-dependent diabetes mellitus” and “non-insulin-dependent diabetes mellitus” and their acronyms “IDDM” and “NIDDM” no longer be used. These terms have been confusing and frequently resulted in patients being classified on the basis of treatment rather than pathogenesis.

- The terms Type 1 and Type 2 should be reintroduced. The aetiological type named Type 1 encompasses the majority of cases which are primarily due to pancreatic islet beta-cell destruction and are prone to ketoacidosis. Type 1 includes those cases attributable to an autoimmune process, as well as those with beta-cell destruction and who are prone to ketoacidosis for which neither an aetiology nor a pathogenesis is known (idiopathic). It does not include those forms of beta-cell destruction or failure to which specific causes can be assigned (e.g. cystic fibrosis, mitochondrial defects, etc.). Some subjects with this type can be identified at earlier clinical stages than “diabetes mellitus”.
- The type named Type 2 includes the common major form of diabetes which results from defect(s) in insulin secretion, almost always with a major contribution from insulin resistance. It has been argued that a lean phenotype of Type 2 diabetes mellitus in adults found in the Indian sub-continent may be very distinct from the more characteristic form of Type 2 found in

Caucasians. Not enough information is available, however, to characterize such subjects separately.

- A recent international workshop reviewed the evidence for, and characteristics of, diabetes mellitus seen in undernourished populations (16,17). Whilst it appears that malnutrition may influence the expression of several types of diabetes, the evidence that diabetes can be caused by malnutrition or protein deficiency *per se* is not convincing. Therefore, it is recommended that the class "Malnutrition-related diabetes" (MRDM) be deleted. The former subtype of MRDM, Protein-deficient Pancreatic Diabetes (PDPD or PDDM), may be considered as a malnutrition modulated or modified form of diabetes mellitus for which more studies are needed. The other former subtype of MRDM, Fibrocalculous Pancreatic Diabetes (FCPD), is now classified as a disease of the exocrine pancreas, fibrocalculous pancreatopathy, which may lead to diabetes mellitus.
- The class "Impaired Glucose Tolerance" is now classified as a stage of impaired glucose regulation, since it can be observed in any hyperglycaemic disorder, and is itself not diabetes.
- A clinical stage of Impaired Fasting Glycaemia has been introduced to classify individuals who have fasting glucose values above the normal range, but below those diagnostic of diabetes.

- Gestational Diabetes is retained but now encompasses the groups formerly classified as Gestational Impaired Glucose Tolerance (GIGT) and Gestational Diabetes Mellitus (GDM).

## **4. Clinical staging of diabetes mellitus and other categories of glucose tolerance (Figure 2)**

### **4.1 Diabetes mellitus**

Diabetes mellitus, regardless of underlying cause, is sub-divided into: *Insulin requiring for survival* (corresponding to the former clinical class of "Insulin Dependent Diabetes Mellitus – IDDM"), e.g. C-peptide deficient; *Insulin requiring for control*, i.e. metabolic control, rather than for survival, e.g. some endogenous insulin secretion but insufficient to achieve normoglycaemia without added exogenous insulin; and *Not insulin requiring*, i.e. those who may be controlled satisfactorily by non-pharmacological methods or drugs other than insulin. Together, the latter two sub-divisions constitute the former class of NIDDM.

### **4.2 Impaired glucose regulation – Impaired Glucose Tolerance (IGT) and Impaired Fasting Glycaemia (IFG)**

Impaired glucose regulation (IGT and IFG) refers to a metabolic state intermediate between normal glucose homeostasis and diabetes. It should be stated unequivocally, however, that IFG and IGT are not interchangeable and represent different abnormalities of glucose regulation, one in the fasting state and one post-prandial.

IGT, rather than being a class as in the previous classification, is categorized as a stage in the natural history of disordered carbohydrate metabolism. A stage of IFG is also recognized because such subjects, like those with IGT, have increased risks of progressing to diabetes and macrovascular disease, although prospective data are sparse and early data suggest a lower risk of progression than IGT (18), although a similar CVD risk factor profile has been shown in IFG and IGT subjects (19). IFG refers to fasting glucose concentrations which are lower than those required to diagnose diabetes mellitus but higher than the "normal" reference range.

The values for IFG are a fasting plasma glucose concentration of  $6.1 \text{ mmol l}^{-1}$  ( $110 \text{ mg dl}^{-1}$ ) or greater (whole blood  $5.6 \text{ mmol l}^{-1}$ ;  $100 \text{ mg dl}^{-1}$ ), but less than  $7.0 \text{ mmol l}^{-1}$  ( $126 \text{ mg dl}^{-1}$ ) (whole blood  $6.1 \text{ mmol l}^{-1}$ ;  $110 \text{ mg dl}^{-1}$ ). If an OGTT is performed, some individuals with IFG will have IGT or diabetes, but this cannot be determined without an OGTT. If resources allow, it is recommended that all those with IFG have an OGTT to exclude the diagnosis of diabetes.

Individuals who meet criteria for IGT or IFG may be euglycaemic in their daily lives as shown by normal or near-normal glycated haemoglobin levels. IGT and IFG are not clinical entities in their own right, but rather risk categories for future diabetes and/or cardiovascular disease (20,21). They can occur as an intermediate stage in any of the disease processes listed in Table 2. IGT is often associated with the Metabolic Syndrome (Insulin Resistance Syndrome) (22). Thus, IGT may not be directly involved in the pathogenesis of cardiovascular disease, but rather may serve as an indicator or marker of enhanced risk by virtue of its correlation with the other elements of the Metabolic

Syndrome that are cardiovascular risk factors. Self-evidently, those individuals with IGT manifest glucose intolerance only when challenged with an oral glucose load.

#### **4.3 Normoglycaemia**

A fasting venous plasma glucose concentration of less than  $6.1 \text{ mmol l}^{-1}$  ( $110 \text{ mg dl}^{-1}$ ) has been chosen as "normal" (Table 1). Although this choice is arbitrary, such values are observed in people with proven normal glucose tolerance, although some may have IGT if an OGTT is performed. Values above this are associated with a progressively greater risk of developing micro- and macrovascular complications (8,9,21,23).

The pathological or aetiological processes which often lead to diabetes mellitus begin, and may be recognizable, in some subjects who have normal glucose tolerance. Recognition of the pathological process at an early stage may be useful if progression to more advanced stages can be prevented. Conversely, effective treatments, or occasionally the natural history of some forms of diabetes mellitus, may result in reversion of hyperglycaemia to a state of normoglycaemia. The proposed classification includes a stage of normoglycaemia in which persons who have evidence of the pathological processes which may lead to diabetes mellitus, or in whom a reversal of the hyperglycaemia has occurred, are classified.

## **5. Aetiological types**

*(see also section 7 and Table 2)*

The aetiological types designate defects, disorders or processes which often result in diabetes mellitus.

### **5.1 Type 1**

Type 1 indicates the processes of beta-cell destruction that may ultimately lead to diabetes mellitus in which "insulin is required for survival" to prevent the development of ketoacidosis, coma and death. An individual with a Type 1 process may be metabolically normal before the disease is clinically manifest, but the process of beta-cell destruction can be detected. Type 1 is usually characterized by the presence of anti-GAD, islet cell or insulin antibodies which identify the autoimmune processes that lead to beta-cell destruction. In some subjects with this clinical form of diabetes, particularly non-Caucasians, no evidence of an autoimmune disorder is demonstrable and these are classified as "Type 1 idiopathic". Aetiological classification may be possible in some circumstances and not in others. Thus, the aetiological Type 1 process can be identified and sub-categorized if appropriate antibody determinations are performed. It is recognized that such measurements may be available only in certain centres at the present time. If these measurements are performed, then the classification of individual patients should reflect this.

## **5.2 Type 2**

Type 2 is the most common form of diabetes and is characterized by disorders of insulin action and insulin secretion, either of which may be the predominant feature. Both are usually present at the time that this form of diabetes is clinically manifest. By definition, the specific reasons for the development of these abnormalities are not yet known.

## **5.3 Other specific types (Table 3)**

Other specific types are currently less common causes of diabetes mellitus, but are those in which the underlying defect or disease process can be identified in a relatively specific manner. They include, for example, fibrocalculous pancreatopathy, a form of diabetes which was formerly classified as one type of malnutrition-related diabetes mellitus.

## **6. Gestational Hyperglycaemia and Diabetes**

Gestational diabetes is carbohydrate intolerance resulting in hyperglycaemia of variable severity with onset or first recognition during pregnancy. It does not exclude the possibility that the glucose intolerance may antedate pregnancy but has been previously unrecognized. The definition applies irrespective of whether or not insulin is used for treatment or the condition persists after pregnancy.

Women who become pregnant and who are known to have diabetes mellitus which antedates pregnancy do not have gestational diabetes but have "diabetes mellitus and pregnancy" and should be treated accordingly before, during, and after the pregnancy.

In the early part of pregnancy (e.g. first trimester and first half of second trimester) fasting and postprandial glucose concentrations are normally lower than in normal, non-pregnant women. Elevated fasting or postprandial plasma glucose levels at this time in pregnancy may well reflect the presence of diabetes which has antedated pregnancy, but criteria for designating abnormally high glucose concentrations at this time have not yet been established. The occurrence of higher than usual plasma glucose levels at this time in pregnancy mandates careful management and may be an indication for carrying out an OGTT (Annex 1). Nevertheless, normal glucose tolerance in the early part of

pregnancy does not itself establish that gestational diabetes may not develop later.

Individuals at high risk for gestational diabetes include older women, those with previous history of glucose intolerance, those with a history of large for gestational age babies, women from certain high-risk ethnic groups, and any pregnant woman who has elevated fasting, or casual, blood glucose levels. It may be appropriate to screen pregnant women belonging to high-risk populations during the first trimester of pregnancy in order to detect previously undiagnosed diabetes mellitus. Formal systematic testing for gestational diabetes is usually done between 24 and 28 weeks of gestation.

### **6.1 Diagnosis of gestational diabetes**

To determine if gestational diabetes is present in pregnant women, a standard OGTT should be performed after overnight fasting (8–14 hours) by giving 75 g anhydrous glucose in 250–300 ml water (Annex 1). Plasma glucose is measured fasting and after 2 hours. Pregnant women who meet WHO criteria for diabetes mellitus or IGT are classified as having Gestational Diabetes Mellitus (GDM). After the pregnancy ends, the woman should be re-classified as having either diabetes mellitus, or IGT, or normal glucose tolerance based on the results of a 75 g OGTT six weeks or more after delivery. It should be emphasized that such women, regardless of the 6-week post-pregnancy result, are at increased risk of subsequently developing diabetes. The significance of IFG in pregnancy remains to be established. Any woman with IFG, however, should have a 75 g OGTT.

## **7. Description of aetiological types**

Patients with any form of diabetes may require insulin treatment at some stage of their disease. Such use of insulin does not, of itself, define the aetiological class.

### **7.1 Type 1 (beta-cell destruction, usually leading to absolute insulin deficiency)**

#### ***7.1.1 Autoimmune Diabetes Mellitus***

This form of diabetes, previously encompassed by the terms insulin-dependent diabetes, Type 1 diabetes, or juvenile-onset diabetes, results from autoimmune mediated destruction of the beta cells of the pancreas. The rate of destruction is quite variable, being rapid in some individuals and slow in others (24). The rapidly progressive form is commonly observed in children, but also may occur in adults (25). The slowly progressive form generally occurs in adults and is sometimes referred to as latent autoimmune diabetes in adults (LADA). Some patients, particularly children and adolescents, may present with ketoacidosis as the first manifestation of the disease (26). Others have modest fasting hyperglycaemia that can rapidly change to severe hyperglycaemia and/or ketoacidosis in the presence of infection or other stress. Still others, particularly adults, may retain residual beta-cell function, sufficient to prevent ketoacidosis, for many years (27). Individuals with this form of Type 1 diabetes often become dependent on insulin for survival eventually and are at risk for ketoacidosis (28). At this stage of the disease, there

is little or no insulin secretion as manifested by low or undetectable levels of plasma C-peptide (29).

Markers of immune destruction, including islet cell autoantibodies, and/or autoantibodies to insulin, and autoantibodies to glutamic acid decarboxylase (GAD) are present in 85–90 % of individuals with Type 1 diabetes mellitus when fasting diabetic hyperglycaemia is initially detected (30). The peak incidence of this form of Type 1 diabetes occurs in childhood and adolescence, but the onset may occur at any age, ranging from childhood to the ninth decade of life (31). There is a genetic predisposition to autoimmune destruction of beta cells, and it is also related to environmental factors that are still poorly defined. Although patients are usually not obese when they present with this type of diabetes, the presence of obesity is not incompatible with the diagnosis. These patients may also have other autoimmune disorders such as Graves' disease, Hashimoto's thyroiditis, and Addison's disease (32).

### **7.1.2 *Idiopathic***

There are some forms of Type 1 diabetes which have no known aetiology. Some of these patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity (33). This form of diabetes is more common among individuals of African and Asian origin. In another form found in Africans an absolute requirement for insulin replacement therapy in affected patients may come and go, and patients periodically develop ketoacidosis (34).

## **7.2 Type 2 (predominantly insulin resistance with relative insulin deficiency or predominantly an insulin secretory defect with/without insulin resistance)**

Diabetes mellitus of this type previously encompassed non-insulin-dependent diabetes, or adult-onset diabetes. It is a term used for individuals who have relative (rather than absolute) insulin deficiency. People with this type of diabetes frequently are resistant to the action of insulin (35,36). At least initially, and often throughout their lifetime, these individuals do not need insulin treatment to survive. This form of diabetes is frequently undiagnosed for many years because the hyperglycaemia is often not severe enough to provoke noticeable symptoms of diabetes (37,38). Nevertheless, such patients are at increased risk of developing macrovascular and microvascular complications (37,38). There are probably several different mechanisms which result in this form of diabetes, and it is likely that the number of people in this category will decrease in the future as identification of specific pathogenetic processes and genetic defects permits better differentiation and a more definitive classification with movement into "Other types". Although the specific aetiologies of this form of diabetes are not known, by definition autoimmune destruction of the pancreas does not occur and patients do not have other known specific causes of diabetes listed in Tables 3–5.

The majority of patients with this form of diabetes are obese, and obesity itself causes or aggravates insulin resistance (39,40). Many of those who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region (41).

Ketoacidosis is infrequent in this type of diabetes; when seen it usually arises in association with the stress of another illness such as infection (42,43). Whereas patients with this form of diabetes may have insulin levels that appear normal or elevated, the high blood glucose levels in these diabetic patients would be expected to result in even higher insulin values had their beta-cell function been normal (44). Thus, insulin secretion is defective and insufficient to compensate for the insulin resistance. On the other hand, some individuals have essentially normal insulin action, but markedly impaired insulin secretion. Insulin sensitivity may be increased by weight reduction, increased physical activity, and/or pharmacological treatment of hyperglycaemia but is not restored to normal (45,46). The risk of developing Type 2 diabetes increases with age, obesity, and lack of physical activity (47,48). It occurs more frequently in women with prior GDM and in individuals with hypertension or dyslipidaemia. Its frequency varies in different racial/ethnic subgroups (47–50). It is often associated with strong familial, likely genetic, predisposition (49–51). However, the genetics of this form of diabetes are complex and not clearly defined.

Some patients who present with a clinical picture consistent with Type 2 diabetes have autoantibodies similar to those found in Type 1 diabetes, and may masquerade as Type 2 diabetes if antibody determinations are not made. Patients who are non-obese or who have relatives with Type 1 diabetes and who are of Northern European origin may be suspected of having late onset Type 1 diabetes.

## **7.3 Other Specific Types**

### *7.3.1 Genetic defects of beta-cell function*

Several forms of the diabetic state may be associated with monogenic defects in beta-cell function, frequently characterized by onset of mild hyperglycaemia at an early age (generally before age 25 years). They are usually inherited in an autosomal dominant pattern. Patients with these forms of diabetes, formerly referred to as maturity-onset diabetes of the young (MODY), have impaired insulin secretion with minimal or no defect in insulin action (52,53). Abnormalities at three genetic loci on different chromosomes have now been characterized. The most common form is associated with mutations on chromosome 12 in a hepatic nuclear transcription factor referred to as HNF1 $\alpha$  (54). A second form is associated with mutations in the glucokinase gene on chromosome 7p (55,56). Glucokinase converts glucose to glucose-6-phosphate, the metabolism of which in turn stimulates insulin secretion by the beta cell. Thus, glucokinase serves as the "glucose sensor" for the beta cell. Because of defects in the glucokinase gene, increased levels of glucose are necessary to elicit normal levels of insulin secretion. A third form is associated with a mutation in the HNF4 $\alpha$  gene on chromosome 20q (57). HNF4 $\alpha$  is a transcription factor which is involved in the regulation of the expression of HNF1 $\alpha$ . A fourth variant has recently been ascribed to mutations in another transcription factor gene, IPF-1, which in its homozygous form leads to total pancreatic agenesis (58).

Specific genetic defects in other individuals who have a similar clinical presentation are currently being defined.

Point mutations in mitochondrial DNA have been found to be associated with diabetes mellitus and deafness (59). The most common mutation occurs at position 3243 in the tRNA leucine gene, leading to an A to G substitution. An identical lesion occurs in the MELAS syndrome (Mitochondrial myopathy, Encephalopathy, Lactic Acidosis, and Stroke-like syndrome); however, diabetes is not part of this syndrome, suggesting for unknown reasons different phenotypic expressions of this genetic lesion (60).

Genetic abnormalities that result in the inability to convert proinsulin to insulin have been identified in a few families. Such traits are usually inherited in an autosomal dominant pattern (61,62) and the resultant carbohydrate intolerance is mild. Similarly, mutant insulin molecules with impaired receptor binding have been identified in a few families. These are also associated with autosomal inheritance and either normal or only mildly impaired carbohydrate metabolism (63,64).

### **7.3.2 Genetic defects in insulin action**

There are some unusual causes of diabetes which result from genetically determined abnormalities of insulin action. The metabolic abnormalities associated with mutations of the insulin receptor may range from hyperinsulinaemia and modest hyperglycaemia to symptomatic diabetes (65,66). Some individuals with these mutations have acanthosis nigricans. Women may have virilization and have enlarged,

cystic ovaries. In the past, this syndrome was termed Type A insulin resistance (65). Leprechaunism and Rabson-Mendenhall syndrome are two paediatric syndromes that have mutations in the insulin receptor gene with subsequent alterations in insulin receptor function and extreme insulin resistance (66). The former has characteristic facial features while the latter is associated with abnormalities of teeth and nails and pineal gland hyperplasia.

#### *7.3.3 Diseases of the exocrine pancreas*

Any process that diffusely injures the pancreas can cause diabetes. Acquired processes include pancreatitis, trauma, infection, pancreatic carcinoma, and pancreatectomy (67,68). With the exception of cancer, damage to the pancreas must be extensive for diabetes to occur. However, adenocarcinomas that involve only a small portion of the pancreas have been associated with diabetes. This implies a mechanism other than simple reduction in beta-cell mass (69). If extensive enough, cystic fibrosis and haemochromatosis will also damage beta cells and impair insulin secretion (70,71). Fibrocalculous pancreatopathy may be accompanied by abdominal pain radiating to the back and pancreatic calcification on X-ray and ductal dilatation (72). Pancreatic fibrosis and calcified stones in the exocrine ducts are found at autopsy.

#### *7.3.4 Endocrinopathies*

Several hormones (e.g. growth hormone, cortisol, glucagon, epinephrine) antagonize insulin action. Diseases associated with excess secretion of these hormones can cause diabetes (e.g. Acromegaly, Cushing's Syndrome, Glucagonoma and

Phaeochromocytoma) (73). These forms of hyperglycaemia typically resolve when the hormone excess is removed.

Somatostatinoma, and aldosteronoma-induced hypokalaemia, can cause diabetes, at least in part by inhibiting insulin secretion (74,75). Hyperglycaemia generally resolves following successful removal of the tumour.

#### *7.3.5 Drug- or chemical-induced diabetes*

Many drugs can impair insulin secretion. These drugs may not, by themselves, cause diabetes but they may precipitate diabetes in persons with insulin resistance (76,77). In such cases, the classification is ambiguous, as the primacy of beta-cell dysfunction or insulin resistance is unknown. Certain toxins such as Vacor (a rat poison) and pentamidine can permanently destroy pancreatic beta cells (78-80). Fortunately, such drug reactions are rare. There are also many drugs and hormones which can impair insulin action. Examples include nicotinic acid and glucocorticoids (71,72). The list shown in Table 4 is not all-inclusive, but reflects the more commonly recognized drug-, hormone-, or toxin-induced forms of diabetes and hyperglycaemia.

#### *7.3.6 Infections*

Certain viruses have been associated with beta-cell destruction. Diabetes occurs in some patients with congenital rubella (81). In addition, Coxsackie B, cytomegalovirus and other viruses (e.g. adenovirus and mumps) have been implicated in inducing the disease (82-84).

### *7.3.7 Uncommon but specific forms of immune-mediated diabetes mellitus*

Diabetes may be associated with several immunological diseases with a pathogenesis or aetiology different from that which leads to the Type 1 diabetes process. Postprandial hyperglycaemia of a severity sufficient to fulfil the criteria for diabetes has been reported in rare individuals who spontaneously develop insulin autoantibodies (85,86). However, these individuals generally present with symptoms of hypoglycaemia rather than hyperglycaemia. The "stiff man syndrome" is an autoimmune disorder of the central nervous system, characterized by stiffness of the axial muscles with painful spasms (87). Affected people usually have high titres of the GAD autoantibodies and approximately one-half will develop diabetes. Patients receiving interferon alpha have been reported to develop diabetes associated with islet cell autoantibodies and, in certain instances, severe insulin deficiency (88).

Anti-insulin receptor antibodies can cause diabetes by binding to the insulin receptor, thereby reducing the binding of insulin to target tissues (89). However, these antibodies also can act as an insulin agonist after binding to the receptor and can thereby cause hypoglycaemia (90). Anti-insulin receptor antibodies are occasionally found in patients with systemic lupus erythematosus and other autoimmune diseases (91). As in other states of extreme insulin resistance, patients with anti-insulin receptor antibodies often have acanthosis nigricans. In the past, this syndrome was termed Type B insulin resistance.

### **7.3.8 Other genetic syndromes sometimes associated with diabetes**

Many genetic syndromes are accompanied by an increased incidence of diabetes mellitus. These include the chromosomal abnormalities of Down's syndrome, Klinefelter's syndrome and Turner's syndrome. Wolfram's syndrome is an autosomal recessive disorder characterized by insulin-deficient diabetes and the absence of beta cells at autopsy (92). Additional manifestations include diabetes insipidus, hypogonadism, optic atrophy, and neural deafness. These and other similar disorders are listed in Table 5.

## **8. The Metabolic Syndrome**

A major classification, diagnostic and therapeutic challenge is the person with hypertension, central (upper body) obesity, and dyslipidaemia, with or without hyperglycaemia. This group of people is at high risk of macrovascular disease (22).

Often a person with abnormal glucose tolerance (IGT or diabetes) will be found to have at least one or more of the other cardiovascular disease (CVD) risk components (22). This clustering has been labelled variously as Syndrome X (22), the Insulin Resistance Syndrome (47), or the Metabolic Syndrome (47).

Epidemiological studies confirm that this syndrome occurs commonly in a wide variety of ethnic groups including Caucasians, Afro-Americans, Mexican-Americans, Asian Indians, Chinese, Australian Aborigines, Polynesians and Micronesians (47,93). In 1988 Reaven focused attention on this cluster, naming it Syndrome X (22). Central obesity was not included in the original description so the term Metabolic Syndrome is now favoured.

Evidence is accumulating that insulin resistance may be the common aetiological factor for the individual components of the Metabolic Syndrome (47,93,94), although there appears to be heterogeneity in the strength of the insulin resistance relationship with different components between, and even within, populations. Alone, each component of the cluster conveys increased CVD risk, but as a combination they

become much more powerful (95). This means that the management of persons with hyperglycaemia and other features of the Metabolic Syndrome should focus not only on blood glucose control but also include strategies for reduction of the other CVD risk factors (96).

It is well documented that the features of the Metabolic Syndrome can be present for up to 10 years before detection of the glycaemic disorders (97). This is important in relation to the aetiology of the hyperglycaemia and the associated CVD risk, and the potential to prevent CVD and its morbidity and mortality in persons with glucose intolerance.

The Metabolic Syndrome with normal glucose tolerance identifies the subject as a member of a group at very high risk of future diabetes. Thus, vigorous early management of the syndrome may have a significant impact on the prevention of both diabetes and cardiovascular disease (98).

### **8.1 Definition**

There is no internationally agreed definition for the Metabolic Syndrome. The following, which does not imply causal relationships, is suggested as a working definition to be improved upon in due course: glucose intolerance, IGT or diabetes mellitus and/or insulin resistance together with two or more of the other components listed below:

- Impaired glucose regulation or diabetes (see Table 1)

- Insulin resistance (under hyperinsulinaemic, euglycaemic conditions, glucose uptake below lowest quartile for background population under investigation)
- Raised arterial pressure  $\geq 140/90$  mmHg
- Raised plasma triglycerides ( $>1.7$  mmol l $^{-1}$ ; 150 mg dl $^{-1}$ ) and/or low HDL-cholesterol ( $< 0.9$  mmol l $^{-1}$ , 35 mg dl $^{-1}$  men;  $< 1.0$  mmol l $^{-1}$ , 39 mg dl $^{-1}$  women)
- Central obesity (males: waist to hip ratio  $> 0.90$ ; females: waist to hip ratio  $> 0.85$ ) and/or BMI  $> 30$  kg m $^{-2}$
- Microalbuminuria (urinary albumin excretion rate  $\geq 20$   $\mu\text{g min}^{-1}$  or albumin:creatinine ratio  $\geq 30$  mg g $^{-1}$ )
- Several other components of the Metabolic Syndrome have been described (e.g. hyperuricaemia, coagulation disorders, raised PAI-1, etc.) but they are not necessary for the recognition of the condition.

## **8.2 Future needs**

A clear description of the essential components of the syndrome is needed together with data to support the relative importance of each component. Internationally agreed criteria for central obesity, insulin resistance and hyperinsulinaemia would be of major assistance.

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## **Annex 1**

### **The Oral Glucose Tolerance Test**

The oral glucose tolerance test (OGTT) is principally used for diagnosis when blood glucose levels are equivocal, during pregnancy, or in epidemiological studies.

The OGTT should be administered in the morning after at least three days of unrestricted diet (greater than 150 g of carbohydrate daily) and usual physical activity. Recent evidence suggests that a reasonable (30–50g) carbohydrate containing meal should be consumed on the evening before the test. The test should be preceded by an overnight fast of 8–14 hours, during which water may be drunk. Smoking is not permitted during the test. The presence of factors that influence interpretation of the results of the test must be recorded (e.g. medications, inactivity, infection, etc.).

After collection of the fasting blood sample, the subject should drink 75 g of anhydrous glucose or 82.5 g of glucose monohydrate (or partial hydrolysates of starch of the equivalent carbohydrate content) in 250–300 ml of water over the course of 5 minutes. For children, the test load should be 1.75 g of glucose per kg body weight up to a total of 75 g of glucose. Timing of the test is from the beginning of the drink. Blood samples must be collected 2 hours after the test load.

Unless the glucose concentration can be determined immediately, the blood sample should be collected in a tube containing sodium fluoride (6 mg per ml whole blood) and immediately centrifuged to separate the plasma; the plasma should be frozen until the glucose concentration can be estimated. For interpretation of results, refer to Table 1.

## **Annex 2**

### **Methods for measuring substances in blood and urine**

#### **Measurement of glucose in blood**

Reductiometric methods (the Somogyi–Nelson, the ferricyanide and neocuprine autoanalyser methods) are still in use for blood glucose measurement. The *o*-toluidine method also remains in use but enzyme-based methods are widely available, for both laboratory and near-patient use. Highly accurate and rapid (1–2 min) devices are now available based on immobilized glucose oxidase electrodes. Hexokinase and glucose dehydrogenase methods are used for reference.

Whole blood samples preserved with fluoride show an initial rapid fall in glucose of up to 10 % at room temperature, but subsequent decline is slow; centrifugation prevents the initial fall. Whole blood glucose values are 15 % lower than corresponding plasma values in patients with a normal haematocrit reading, and arterial values are about 7 % higher than corresponding venous values.

The use of reagent-strip glucose oxidase methods has made bedside estimation of blood glucose very popular. However, the cost of the reagent-strips remains high. Some methods still require punctilious technique, accurate timing, and storage of strips in airtight containers. Reasonably quantitative results can be obtained even with visual colour-matching techniques. Electrochemical and reflectance meters can give coefficients of variation of well under 5 %. Reagent-strip methods have been validated under tropical conditions, but are sensitive to extreme climatic conditions. Diabetes may be strongly suspected from the results of reagent-strip glucose estimation, but the diagnosis cannot be confidently excluded by the use of this method. Confirmation of diagnosis requires estimation by laboratory methods.

Patients can easily collect small blood samples themselves (either in specially prepared plastic or glass capillary tubes or on

filter-paper), and self-monitoring using glucose reagent-strips with direct colour-matching or meters is now widely practised. Patients should be properly trained in the appropriate techniques to avoid inaccurate or misleading results.

The insulin-treated patient is commonly requested to build up a "glycaemic profile" by self-measurement of blood glucose at specific times of the day (and night). A "7-point profile" is useful, with samples taken before and 90 min after breakfast, before and 90 min after lunch, before and 90 min after an evening meal, and just before going to bed. Occasionally patients may arrange to wake at 0300 h to collect and measure a nocturnal sample. The complete profile rarely needs to be collected within a single 24-hour period, and it may be compiled from samples collected at different times over several days.

#### **Measurement of glucose in urine**

Insulin-treated patients who do not have access to facilities for self-measurement of blood glucose should test urine samples passed after rising, before main meals, and before going to bed. Non-insulin-dependent patients do not need to monitor their urine so frequently. Urine tests are of somewhat limited value, however, because of the great variation in urine glucose concentration for given levels of blood glucose. The correlation between blood and urine glucose may be improved a little by collecting short-term fractions (15–30 min) of the urine output. Benedict's quantitative solution or self-boiling, caustic soda/copper sulphate tablets may be used or the more convenient, but costly, semi-quantitative enzyme-based test-strips.

#### **Ketone bodies in urine and blood**

The appearance of persistent ketonuria associated with hyperglycaemia or high levels of glycosuria in the diabetic patient points to an unacceptably severe level of metabolic disturbance and indicates an urgent need for corrective action. The patient should be advised to test for ketone bodies (acetone and aceto-acetic acid) when tests for glucose are

repeatedly positive, or when there is substantial disturbance of health, particularly with infections. Rothera's sodium nitroprusside test may be used or, alternatively, reagent-strips that are sensitive to ketones. In emergency situations such as diabetic ketoacidosis, a greatly raised concentration of plasma ketones can be detected with a reagent-strip and roughly quantified by serial 1 in 2 dilution of plasma with water.

**Table 1: Values for diagnosis of diabetes mellitus and other categories of hyperglycaemia**

	Glucose concentration, mmol l <sup>-1</sup> (mg dL <sup>-1</sup> )		
	Venous	Whole blood	Capillary
<b>Diabetes Mellitus:</b>			
Fasting or 2-h post glucose load	$\geq 6.1 (\geq 110)$	$\geq 6.1 (\geq 110)$	$\geq 7.0 (\geq 126)$
	$\geq 10.0 (\geq 180)$	$\geq 11.1 (\geq 200)$	$\geq 11.1 (\geq 200)$
<b>Impaired Glucose Tolerance (IGT):</b>			
Fasting (if measured) and 2-h post glucose load	$< 6.1 (< 110)$ and $\geq 6.7 (\geq 120)$	$< 6.1 (< 110)$ and $\geq 7.8 (\geq 140)$	$< 7.0 (< 126)$ and $\geq 7.8 (\geq 140)$
<b>Impaired Fasting Glycaemia (IFG):</b>			
Fasting and (if measured)	$\geq 5.6 (\geq 100)$ and $< 6.1 (< 110)$	$\geq 5.6 (\geq 100)$ and $< 6.1 (< 110)$	$\geq 6.1 (\geq 110)$ and $< 7.0 (< 126)$
2-h post glucose load	$< 6.7 (< 120)$	$< 7.8 (< 140)$	$< 7.8 (< 140)$

\* Corresponding values for capillary plasma are: for Diabetes Mellitus, fasting  $\geq 7.0 (\geq 126)$ , 2-h  $\geq 12.2 (\geq 220)$ ; for Impaired Glucose Tolerance, fasting  $< 7.0 (< 126)$  and 2-h  $\geq 8.9 (\geq 160)$  and  $< 12.2 (\geq 220)$ ; and for Impaired Fasting Glycaemia  $\geq 6.1 (\geq 110)$  and  $> 7.0 (< 126)$  and if measured, 2-h  $< 8.9 (< 160)$ .

For epidemiological or population screening purposes, the fasting or 2-h value after 75 g oral glucose may be used alone. For clinical purposes, the diagnosis of diabetes should always be confirmed by repeating the test on another day unless there is unequivocal hyperglycaemia with acute metabolic decompensation or obvious symptoms.

Glucose concentrations should not be determined on serum unless red cells are immediately removed, otherwise glycolysis will result in an unpredictable under-estimation of the true concentrations. It should be stressed that glucose preservatives do not totally prevent glycolysis. If whole blood is used, the sample should be kept at 0–4 °C or centrifuged immediately, or assayed immediately.

**Table 2. Aetiological Classification of Disorders of Glycaemia\***

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**Type 1 (beta-cell destruction, usually leading to absolute insulin deficiency)**

Autoimmune  
Idiopathic

**Type 2 (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with or without insulin resistance)**

**Other specific types (see Table 3)**

Genetic defects of beta-cell function  
Genetic defects in insulin action  
Diseases of the exocrine pancreas  
Endocrinopathies  
Drug- or chemical-induced  
Infections  
Uncommon forms of immune-mediated diabetes  
Other genetic syndromes sometimes associated with diabetes

**Gestational diabetes\*\***

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\*As additional subtypes are discovered it is anticipated that they will be reclassified within their own specific category.

\*\*Includes the former categories of gestational impaired glucose tolerance and gestational diabetes.

**Table 3. Other Specific Types of Diabetes**

**Genetic defects of beta-cell function**

- Chromosome 20, HNF4 $\alpha$  (MODY1)
- Chromosome 7, glucokinase (MODY2)
- Chromosome 12, HNF1 $\alpha$  (MODY3)
- Chromosome 13, IPF-1 (MODY4)
- Mitochondrial DNA 3243 mutation
- Others

**Genetic defects in insulin action**

- Type A insulin resistance
- Leprechaunism
- Rabson-Mendenhall syndrome
- Lipoatrophic diabetes
- Others

**Diseases of the exocrine pancreas**

- Fibrocalculous pancreatopathy
- Pancreatitis
- Trauma / pancreatectomy
- Neoplasia
- Cystic fibrosis
- Haemochromatosis
- Others

**Endocrinopathies**

- Cushing's syndrome
- Acromegaly
- Phaeochromocytoma
- Glucagonoma
- Hyperthyroidism
- Somatostatinoma
- Others

*(Continued on page 55)*

**Table 3 (continued)**

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**Drug- or chemical-induced (see Table 4)**

**Infections**

Congenital rubella  
Cytomegalovirus  
Others

**Uncommon forms of immune-mediated diabetes**

Insulin autoimmune syndrome (antibodies to insulin)  
Anti-insulin receptor antibodies  
"Stiff Man" syndrome  
Others

**Other genetic syndromes (see Table 5)**

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**Table 4. Drug- or Chemical-induced Diabetes**

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Nicotinic acid  
Glucocorticoids  
Thyroid hormone  
Alpha-adrenergic agonists  
Beta-adrenergic agonists  
Thiazides  
Dilantin  
Pentamidine  
Vacor  
Interferon-alpha therapy  
Others

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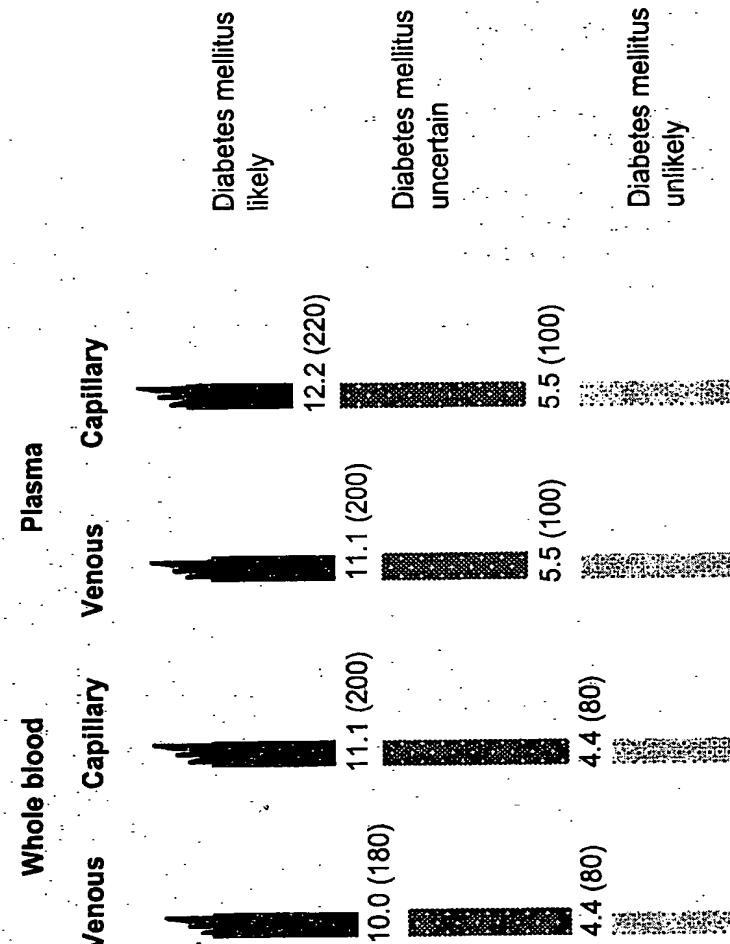
**Table 5. Other Genetic Syndromes Sometimes Associated with Diabetes**

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Down's syndrome  
Friedreich's ataxia  
Huntington's chorea  
Klinefelter's syndrome  
Lawrence-Moon-Biedel syndrome  
Myotonic dystrophy  
Porphyria  
Prader-Willi syndrome  
Turner's syndrome  
Wolfram's syndrome  
Others

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**Figure 1:** Unstandardized (casual, random) blood glucose values in the diagnosis of diabetes in mmol l<sup>-1</sup> (mg dl<sup>-1</sup>). Taken from the 1985 WHO Study Group Report (3).



**Figure 2:** Disorders of glycaemia: aetiological types and clinical stages.

Types	Stages	Normoglycaemia			Hyperglycaemia		
		Normal glucose tolerance	Impaired glucose regulation IGT and/or IFG	Diabetes Mellitus			
				Not insulin requiring	Insulin requiring for control	Insulin requiring for survival	
Type 1							→
• Autoimmune • Idiopathic							
Type 2*							→
• Predominantly insulin resistance							→
• Predominantly insulin secretory defects							
Other specific types*							→
Gestational diabetes*							→
							→

\* In rare instances patients in these categories (e.g. Vacor Toxicity, Type 1 presenting in pregnancy, etc.) may require insulin for survival.

## Increased Susceptibility to Streptozotocin-Induced $\beta$ -Cell Apoptosis and Delayed Autoimmune Diabetes in Alkylpurine-DNA-N-Glycosylase-Deficient Mice

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Type 1 diabetes is thought to occur as a result of the loss of insulin-producing pancreatic  $\beta$  cells by an environmentally triggered autoimmune reaction. In rodent models of diabetes, streptozotocin (STZ), a genotoxic methylating agent that is targeted to the  $\beta$  cells, is used to trigger the initial cell death. High single doses of STZ cause extensive  $\beta$ -cell necrosis, while multiple low doses induce limited apoptosis, which elicits an autoimmune reaction that eliminates the remaining cells. We now show that in mice lacking the DNA repair enzyme alkylpurine-DNA-N-glycosylase (APNG),  $\beta$ -cell necrosis was markedly attenuated after a single dose of STZ. This is most probably due to the reduction in the frequency of base excision repair-induced strand breaks and the consequent activation of poly(ADP-ribose) polymerase (PARP), which results in catastrophic ATP depletion and cell necrosis. Indeed, PARP activity was not induced in APNG<sup>−/−</sup> islet cells following treatment with STZ in vitro. However, 48 h after STZ treatment, there was a peak of apoptosis in the  $\beta$  cells of APNG<sup>−/−</sup> mice. Apoptosis was not observed in PARP-inhibited APNG<sup>+/+</sup> mice, suggesting that apoptotic pathways are activated in the absence of significant numbers of DNA strand breaks. Interestingly, STZ-treated APNG<sup>−/−</sup> mice succumbed to diabetes 8 months after treatment, in contrast to previous work with PARP inhibitors, where a high incidence of  $\beta$ -cell tumors was observed. In the multiple-low-dose model, STZ induced diabetes in both APNG<sup>−/−</sup> and APNG<sup>+/+</sup> mice; however, the initial peak of apoptosis was 2.5-fold greater in the APNG<sup>−/−</sup> mice. We conclude that APNG substrates are diabetogenic but by different mechanisms according to the status of APNG activity.

Type 1 diabetes occurs in a genetically susceptible human population as a result of the loss of the insulin-producing pancreatic  $\beta$  cells. The disease is thought to be triggered by an environmental agent(s) that initiates processes leading to an eventual  $\beta$ -cell-destructive autoimmune response (6, 12, 14). In animal models of the disease, the first observable abnormality is an initial low level of  $\beta$ -cell death (27, 28) that primes antigen-presenting cells such as dendritic cells and macrophages (19). This leads to the proliferation of autoreactive lymphocytes and the ensuing selective elimination of the remaining  $\beta$  cells.

Much of what is known about the cellular mechanisms leading to type 1 diabetes has come from the study of both the nonobese diabetic (NOD) mouse and the use of the methylating agent streptozotocin [2-deoxy-2-(3-methyl-3-nitrosourea)-1-D-glucopyranose] (STZ) as the environmental trigger for the disease. STZ is actively transported into pancreatic  $\beta$  cells via the Glut-2 glucose transporter. It reacts at many sites in DNA but in particular at the ring nitrogen and exocyclic oxygen atoms of the DNA bases, predominantly producing 7-methylguanine, 3-methyladenine (3-meA), and O<sup>6</sup>-methylguanine adducts (1, 37). 3-meA and 7-methylguanine are removed by the

action of alkylpurine-DNA-N-glycosylase (APNG) (also referred to as 3-methyladenine DNA glycosylase), leaving an apurinic/apyrimidinic (AP) site that is acted upon by an AP endonuclease. The resulting DNA strand breaks activate poly(ADP-ribose) polymerase (PARP), which synthesizes polymers of ADP-ribose from NAD<sup>+</sup>, modifying acceptor proteins at the site of DNA damage (38). PARP is thus part of a protein complex that includes XRCC1, DNA polymerase  $\beta$ , and DNA ligase III and is required for the efficient resynthesis and ligation steps of base excision repair (5).

Therefore, a single high dose of STZ will produce a large number of DNA strand breaks, leading to the overactivation of PARP. This results in a catastrophic fall in cellular NAD<sup>+</sup> levels and thus to nonphysiological concentrations of ATP, loss of membrane integrity, and necrotic  $\beta$ -cell death (30). In agreement with this, mice deficient in PARP or treated with PARP inhibitors are protected from STZ-induced  $\beta$ -cell necrosis and do not develop hyperglycemia (2, 25, 33). However, although the initial destruction of the  $\beta$  cells can be avoided by the use of PARP inhibitors, the resulting long-term biological consequence of this is the development of a high incidence of pancreatic  $\beta$ -cell tumors (36, 40). Conversely, a regimen of five daily subdiabetogenic doses of STZ (the multiple-low-dose STZ model, MLDS) induces a peak of apoptotic  $\beta$ -cell death after 5 days of STZ treatment (27), while a second peak of apoptosis is seen at 11 days, when lymphocytic infiltration of

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the islet occurs. The preimmune stage of  $\beta$ -cell apoptosis has also been reported to occur in the NOD mouse model (28). However, the molecular mechanisms leading to the initial peak of STZ-induced  $\beta$ -cell apoptosis in the MLDS model are unclear, and it is also unknown whether the initial induction of  $\beta$ -cell apoptosis is important in triggering the resulting autoimmune reaction.

Thus, while the role of PARP in STZ-induced  $\beta$ -cell death has been studied extensively, we were interested in determining the role of APNG in modulating both the cytotoxic effects of a single high dose of STZ and its effect in the MLDS model of diabetes. Using a recently described APNG-deficient mouse strain (8), our results showed that although APNG<sup>+/+</sup> mice were substantially resistant to single, high-dose STZ-induced  $\beta$ -cell necrosis and the initial onset of diabetes, a smaller peak of  $\beta$ -cell apoptosis was observed 48 h after treatment. However, analogous to the onset of autoimmune diabetes in the NOD mouse model but in contrast to that reported for PARP-inhibited animals, the STZ-treated APNG<sup>+/+</sup> mice eventually succumbed to diabetes after several months due to a marked autoimmune reaction. Additionally, in the MLDS model of diabetes, the APNG<sup>+/+</sup> mice also exhibited an initial increased sensitivity to  $\beta$ -cell apoptosis.

#### MATERIALS AND METHODS

**Mice.** APNG<sup>+/+</sup> mice were generated as previously described (8). They were backcrossed onto a C57BL/6J background for nine generations, and the APNG<sup>+/+</sup> mice thus generated were crossed to yield the male mice used in these experiments. All animal experiments were carried out under the Animals (Scientific Procedures) Act 1986, in the United Kingdom.

**Animal dosing.** STZ (Sigma) was dissolved in sodium citrate buffer (pH 4.5) and injected intraperitoneally, either as a single high dose of 140 mg/kg or, for the MLDS experiments, as five daily doses of 40 mg/kg. In some experiments, 30 min before STZ treatment, 3-aminobenzamide (3-ab; Sigma) prepared in 0.9% saline was administered by intravenous injection at a dose of 340 mg/kg of body weight. Control animals were similarly injected with vehicle only.

**Determination of pancreatic insulin and glucose levels.** Pancreata were dissected; either they were placed entirely in 10 ml of acidified ethanol, or they were divided longitudinally and one half was placed in acidified ethanol while the other was prepared for histological examination. For insulin extraction, the organ was cut into small pieces and then sonicated twice for 30 s at 216  $\mu$ m (peak-to-peak amplitude; Heat Systems). The sonicated material was kept at 4°C for 16 h to extract the insulin and then aliquoted for storage at -20°C. The insulin concentration was measured by radioimmunoassay, using rat insulin as the standard as previously described (15). The blood glucose level was determined by reflectance photometry (Glucotrend blood glucose meter; Boehringer Mannheim) in conjunction with oxidoreductase reaction strips (Glucotrend glucose strips; Roche).

**Histological examination of pancreata.** Mice were sacrificed at 8, 12, 24, 48, and 96 h after STZ treatment. At autopsy, the pancreata were fixed in 4% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.2), processed for paraffin embedding, sectioned (3  $\mu$ m), and stained with hematoxylin-eosin. The percentage of morphologically abnormal  $\beta$  cells was calculated by scoring 500 to 1,000 islet cells per animal and was used as an indication of  $\beta$ -cell death. Scoring  $\beta$ -cell apoptosis using  $\beta$ -cell-specific stains was not appropriate since the cytoplasmic and nuclear morphology was not good enough when these methods were used. Since necrotic and apoptotic nuclei are smaller than normal nuclei, the morphological scoring can be regarded as only semiquantitative. Therefore, apoptosis and necrosis was confirmed by transmission electron microscopy. Mice were perfused with 4% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.2), and 1- by 3-mm slices of pancreas were transferred to 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for 3 h, resin embedded, and stained for electron microscopy as previously described (20).

**Immunohistochemistry.** (I) Insulin. Formalin-fixed pancreata were embedded in paraffin, and 3- $\mu$ m-thick sections were cut and mounted onto 3-aminopropyltriethoxysilane-coated slides. After being dewaxed through xylene and absolute ethanol, the slides were rehydrated through decreasing concentrations of ethanol

(100, 90, 70, and 40%) and rinsed in distilled water. The slides were washed thoroughly in Tris-buffered saline (pH 7.5) (TBS), and endogenous peroxidase was blocked by incubation with 3% H<sub>2</sub>O<sub>2</sub> in TBS for 20 min. The slides were again washed in water and TBS and incubated with 5% normal rabbit serum for 20 min, before being exposed to the primary antibody, a polyclonal guinea pig anti-swine insulin (1:4 dilution; Dako) at 4°C overnight. After being washed in TBS, the slides were incubated with the secondary antibody, biotinylated rabbit anti-guinea pig immunoglobulins (1:200 dilution; Dako) for 30 min at room temperature, washed in TBS, and incubated with an avidin-biotin-horseradish peroxidase complex (Dako) for 40 min at room temperature. The antibody-antigen complexes were visualized by 3,3-diaminobenzidine (Sigma) staining for 5 min. Finally, the slides were counterstained with hematoxylin, dehydrated, and mounted.

(II) T-cell markers CD4 and CD8. Pancreata were snap frozen in liquid nitrogen, and 4- to 5- $\mu$ m sections were prepared. The sections were then fixed in cold acetone for 10 min, air dried, and washed in TBS. Endogenous peroxidase was blocked as above. The sections were incubated with 5% normal rabbit serum for 20 min and then exposed overnight to one of the primary antibodies, either rat anti-mouse CD4<sup>+</sup>, or rat anti-mouse CD8<sup>+</sup> (BD Pharmingen), at 2  $\mu$ g/ml. After being washed in TBS, the sections were incubated with biotinylated rabbit anti-rat immunoglobulins (1:300 dilution; Dako) for 30 min at room temperature. Treatment with avidin-biotin-horseradish peroxidase and then 3,3-diaminobenzidine was as used for the detection of insulin.

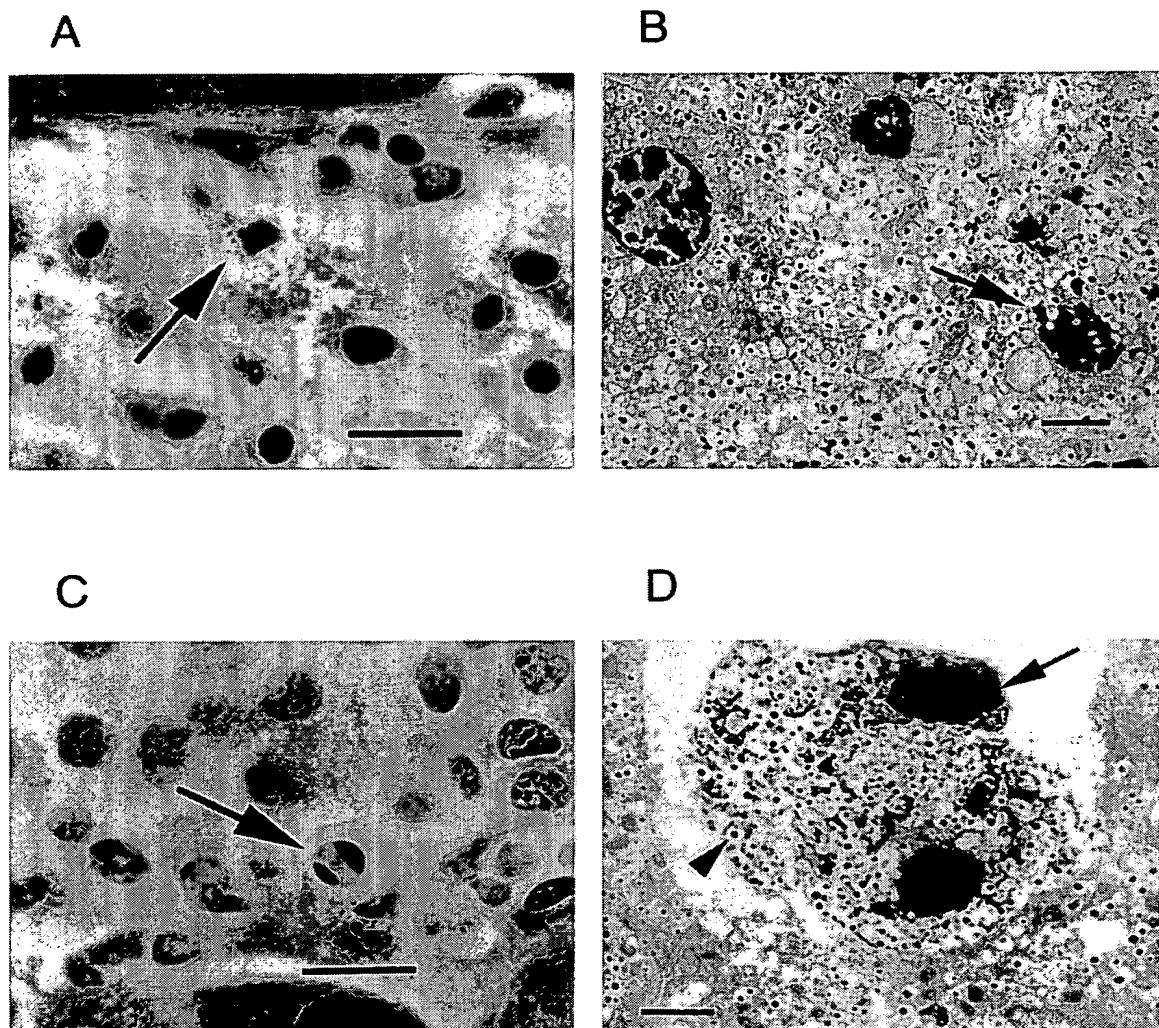
**Isolation of pancreatic islets and treatment with STZ.** Islets were isolated using the method of Lake et al. (22). Using this method, 150 to 200 islets could be reliably obtained. Groups of 150 freshly isolated islets (one animal) were incubated for 30 min in 2.2 mM STZ in Ham's F-10 medium (Life Technologies) or in medium only.

**Isolation of islet nuclei and estimation of PARP activity.** PARP activity in isolated islets was measured essentially as previously described (3). Briefly, following STZ treatment, the islets were washed in Ham's F-10 medium and resuspended in a solution containing 250 mM sucrose, 10 mM HEPES (pH 7.4), 2.5 mM EDTA, 2 mM cysteine, and 0.02% bovine serum albumin. The islets were left on ice for 5 min to lyse and then dispersed by rapid pipetting. The nuclei were pelleted by centrifugation at 1,000  $\times$  g for 3 min and resuspended in 100  $\mu$ l of buffer (50 mM Tris-HCl [pH 7.5], 30% glycerol, 1 mM EDTA, 0.5 mM EGTA) and added to an equal volume of 100 mM Tris-HCl (pH 8.0)-20 mM 2-mercaptoethanol-10 mM MgCl<sub>2</sub> containing 5  $\mu$ Ci of [2,5,8-<sup>3</sup>H]NAD (Amersham Pharmacia Biotech). The mixture was vortexed briefly and incubated for 30 min at 37°C; then 1 ml of ice-cold stop solution (10% trichloroacetic acid, 2% sodium pyrophosphate decahydrate) was added. The samples were left on ice for 45 min and then centrifuged at 12,000  $\times$  g for 10 min. The supernatant was aspirated, and the pellet was washed three more times with 1 ml of stop solution and then once in 0.6 M perchloric acid. The pellet resuspended and left overnight in 200  $\mu$ l of 0.04 N NaOH. A portion (150  $\mu$ l) of the solution was added to a scintillant, and the amount of radioactivity present was measured in a scintillation counter. The remainder of the solution was used to measure DNA content as previously described (21). Results are expressed as femtmoles of NAD<sup>+</sup> incorporated per minute per microgram of DNA.

**Statistical analysis.** The results obtained with groups from each study were first analyzed using analysis of variance. Groups that showed differences were further analyzed by Student's *t* test.

#### RESULTS

**Effect of APNG deletion on  $\beta$ -cell survival following a single high dose of STZ.** To test our hypothesis that APNG-deleted mice should be resistant to STZ-induced necrosis of pancreatic  $\beta$  cells, normal mice (APNG<sup>+/+</sup>) and mice heterozygous (APNG<sup>+/−</sup>) or homozygous (APNG<sup>−/−</sup>) for the APNG null mutation were injected with a single dose of STZ (140 mg/kg) and scored for islet cell morphology by light microscopy. Under light microscopy, necrotic islet cells could be easily identified by their pycnotic nuclei and fragmented cytoplasm (Fig. 1A). For the three mouse strains, the difference in the number of necrotic islet cells after treatment was striking (Fig. 2A). At 8 h after the STZ dose, 60% of the cells from normal mice showed nuclear pycnosis whereas fewer than 5% of the cells from APNG<sup>−/−</sup> mice did so. In normal mice, the number of



**FIG. 1.** Light and electron microscopy of sections of pancreatic islets at 8 and 48 h after the STZ dose (140 mg/kg). (A and B) Light microscopy of islet cells from APNG<sup>+/+</sup> mice reveals extensive necrosis with fragmented nuclei (arrow) at 8 h postdose (A), while electron microscopy shows nuclei clumped with ill-defined edges (arrow) and karyolysis, indicative of necrosis (B). (C) In contrast, light microscopy of islets from APNG<sup>-/-</sup> mice 48 h postdose showed increased numbers of apoptotic islet cells with shrunken cytoplasm and intact nuclear and cytoplasmic membranes. Nuclei were clumped into well-defined masses marginated against the nuclear membrane (arrow). (D) Electron microscopy of the APNG<sup>-/-</sup> STZ-treated islets showed cells with condensed nuclear bodies (arrow), intact membranes, and condensed cytoplasm containing insulin granules (arrowhead), indicative of an apoptotic β cell. Magnification,  $\times 400$  (A and C); bar, 10  $\mu\text{m}$ ;  $\times 2,800$  (B and D); bar, 2  $\mu\text{m}$ .

islet cells containing pycnotic nuclei was reduced to background levels over the next 2 days. No delayed increase in nuclear pycnosis was observed in the islets from APNG<sup>-/-</sup> mice (Fig. 2A). Interestingly, there was a gene dosage effect, with islet cells from APNG<sup>+/+</sup> mice exhibiting approximately half the normal level of nuclear pycnosis at 8, 12, and 24 h postdose, although this was statistically significant only for the 8- and 12-h values.

The primary mode of cell death for islet cells from STZ-treated APNG<sup>+/+</sup> mice was confirmed to be necrosis by transmission electron microscopy (Fig. 1B). The necrotic β cells displayed clumping of the chromatin with ill-defined edges and karyolysis, while the mitochondria were swollen. At 24 h post-

dose, most of the β cells were dead, leaving an area of necrosis at the center of the islets. In contrast, the islets of APNG<sup>-/-</sup> mice at 8 h postdose contained only an occasional necrotic β cell. However, in APNG<sup>-/-</sup> mice and to a lesser extent in APNG<sup>+/+</sup> mice, significant numbers of apoptotic islet cells were seen from 24 h, with a peak at 48 h postdose (Fig. 2B). Under light microscopy, hematoxylin-and-eosin-stained apoptotic β cells could be identified by their chromatin morphology, size, and cytoplasmic staining (Fig. 1C). Apoptotic nuclei were either clumped and marginated or fragmented into regularly shaped membrane-bound bodies. Apoptotic cells appeared shrunken and more densely eosinophilic. Apoptosis was confirmed by transmission electron microscopy; apoptotic

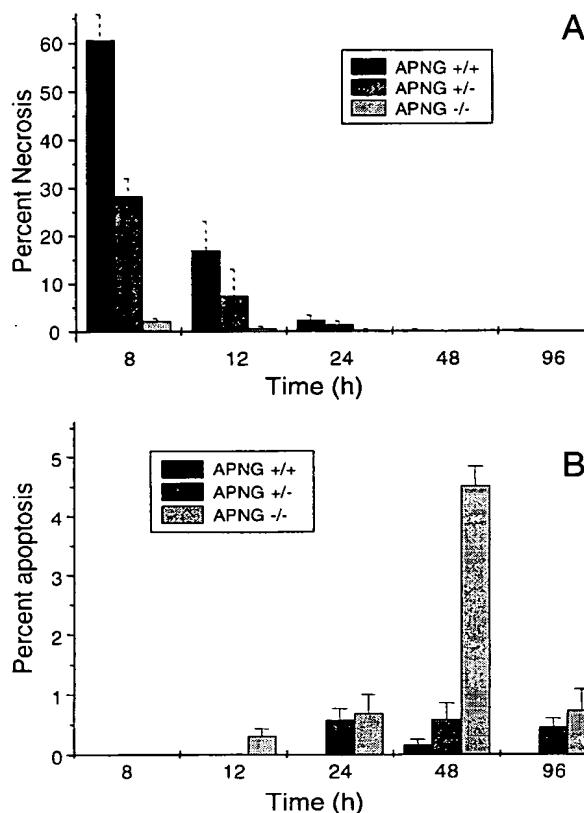


FIG. 2. Proportion of islet cells showing nuclear pycnosis consistent with necrosis (A) and islet cell apoptosis (B) in APNG<sup>+/+</sup>, APNG<sup>+/-</sup>, and APNG<sup>-/-</sup> mice 8 to 96 h after a single injection of STZ (140 mg/kg). Each data point represents the mean and standard error of the mean for 500 islet cells from four animals.

cells appeared shrunken but retained intact nuclear and cell membranes (Fig. 1D). Nuclear chromatin was clumped into well-defined masses marginated against the nuclear membrane or in separate membrane-bound bodies. Apoptotic islet cells stained positive for insulin, confirming that they were  $\beta$  cells (data not shown).

**Effect of APNG deletion on PARP activation.** To confirm that APNG deletion attenuated the activation of PARP, thereby maintaining cellular ATP levels and preventing the initial catastrophic necrosis, pancreatic islets were isolated from APNG<sup>+/+</sup> and APNG<sup>-/-</sup> mice and treated with STZ in vitro. Measurement of PARP activity made by the incorporation of [<sup>3</sup>H]NAD<sup>+</sup> into poly(ADP-ribose) polymers showed that APNG<sup>-/-</sup> islets had significantly less PARP activity after STZ treatment than did APNG<sup>+/+</sup> islets ( $P < 0.01$ ) and were not significantly different from untreated controls ( $P = 0.19$ ) (Fig. 3). Therefore, since DNA strand breaks are required for PARP activation, this result indicates that the bulk of the STZ-induced DNA base adducts were not repaired by base excision in the absence of APNG. It is reasonable to suggest that the persistence of at least a subset of these adducts could be responsible for the apoptotic response observed in the APNG<sup>-/-</sup> mice.

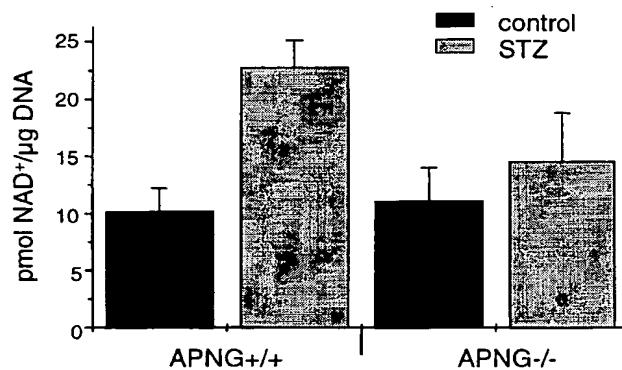


FIG. 3. PARP activity in islets isolated from APNG<sup>+/+</sup> and APNG<sup>-/-</sup> mice. PARP activity was measured in islet cells following incubation for 30 min in 2.2 mM STZ or medium alone. While PARP activity was significantly increased in STZ-treated islets from APNG<sup>+/+</sup> mice compared to untreated controls ( $P < 0.001$ ), there was no significant difference in PARP activity between STZ-treated and control islets from APNG<sup>-/-</sup> mice. Error bars indicated the mean  $\pm$  standard deviation.

**Effect of APNG deletion on high-dose-STZ-induced diabetes.** The pancreatic insulin content was measured at 8 and 96 h to give an indication of the extent of pancreatic  $\beta$ -cell destruction after a single high dose of STZ. Figure 4 shows that at 8 h, pancreatic insulin levels in both APNG<sup>+/+</sup> and APNG<sup>-/-</sup> mice were similar. By 96 h, the pancreatic insulin content in APNG<sup>+/+</sup> mice had fallen by 90% to around 4 ng of insulin per mg of pancreas whereas the levels in STZ-treated APNG<sup>-/-</sup> mice were reduced by only 50% of the control level (Fig. 4). These results corroborate the observed histological findings and confirm that the insulin-secreting  $\beta$  cells are the apoptotic cells of the APNG<sup>-/-</sup> islets. Since previous reports had shown that PARP-deficient mice were also resistant to STZ-induced diabetes (2, 25, 33), we were interested in knowing the effect of the PARP inhibitor 3-ab on diabetes induction in the APNG<sup>-/-</sup> mice. Pretreatment of normal mice with 3-ab (340

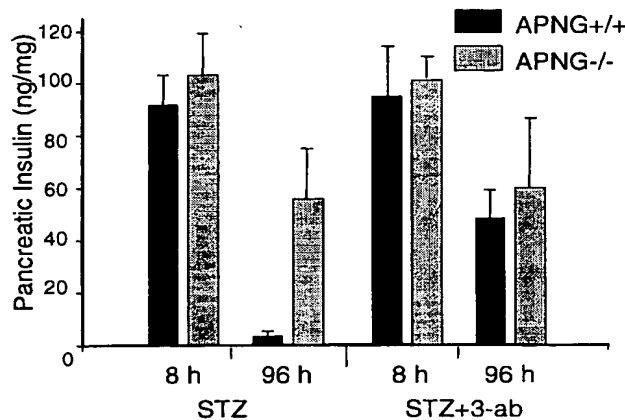


FIG. 4. Effect of pretreatment with 3-ab on pancreatic insulin levels of APNG<sup>+/+</sup> and APNG<sup>-/-</sup> mice, 8 and 96 h after treatment with a single dose of STZ (140 mg/kg). Error bars indicate the mean  $\pm$  the standard error of the mean.

TABLE 1. Effect of 3-ab on high-dose-STZ-induced  $\beta$ -cell necrosis at 8 h and apoptosis at 48 h.

Mouse strain	3-ab	% Necrosis at 8 h <sup>a</sup> (no. of mice)	% Apoptosis at 48 h <sup>a</sup> (no. of mice)
APNG <sup>+/+</sup>	—	73 ± 3.0 (5)	0.25 ± 0.16 (4)
	+	0.5 ± 0.3 (5)	0.87 ± 0.26 (4)
APNG <sup>-/-</sup>	—	2.1 ± 0.7 (5)	4.3 ± 1.0 (4)
	+	0.2 ± 0.1 (5)	2.63 ± 0.56 (5)

<sup>a</sup> For apoptosis values,  $P < 0.01$  for both comparisons between APNG<sup>+/+</sup> and APNG<sup>-/-</sup> mice. For necrosis and apoptosis values, data are expressed as the mean ± the standard error of the mean.

mg/kg) substantially protected them against a fall in pancreatic insulin levels at 96 h (Fig. 4), in agreement with the previous reports. However, pretreatment with 3-ab had no effect on the insulin levels of STZ-treated APNG<sup>-/-</sup> mice at 96 h (Fig. 4). Indeed, there was no significant difference between the pancreatic insulin levels in 3-ab-pretreated APNG<sup>+/+</sup> and APNG<sup>-/-</sup> mice and APNG<sup>-/-</sup> mice treated with STZ alone. Since PARP acts at the DNA strand breaks arising from the action of APNG and AP-endonuclease, these results indicate that the APNG-deleted cells survive because of the absence of DNA strand scission and that any inhibition of a later step in the pathway has no effect on cell survival.

To further compare the mechanisms by which the lack of APNG or PARP leads to cell survival in this system, we investigated the effect of 3-ab on islet cell morphology at 8 and 48 h after STZ treatment in APNG<sup>+/+</sup> and APNG<sup>-/-</sup> mice. As expected, histological analysis at 8 h showed that 3-ab-pretreated APNG<sup>+/+</sup> mice displayed considerably less  $\beta$ -cell necrosis than did those treated with STZ alone ( $P < 0.001$ ) (Table 1). Similarly, PARP inhibition in APNG<sup>-/-</sup> mice resulted in a small but significant reduction in islet cell necrosis ( $P < 0.05$ ) (Table 1). This most probably reflects a low level of strand break induction by nonenzymatic depurination and glycosylase-catalyzed removal of STZ-induced oxidative base damage to the DNA in the APNG<sup>-/-</sup> mice. For apoptosis at 48 h, it is clear from Table 1 that 3-ab treatment of APNG<sup>+/+</sup> mice did not produce a peak of apoptosis similar to that found in APNG<sup>-/-</sup> mice. The inhibition of PARP had no significant effect on the peak of apoptosis observed in APNG<sup>-/-</sup> islets ( $P = 0.15$ ) (Table 1). These results suggest that it is the persistence of DNA adducts that act as a signal for the cell to undergo apoptosis.

**Induction of diabetes in APNG<sup>-/-</sup> mice after a single high dose of STZ.** To assess the long-term effects of high-dose STZ treatment in APNG<sup>-/-</sup> mice, STZ-treated animals were monitored for general well-being over several months. At approximately 8 months postdose, all the mice were diabetic, with fasting blood glucose levels averaging above 10 mM and significantly reduced pancreatic insulin levels compared to equivalently aged untreated controls (Table 2). Several mice also exhibited marked lipolysis, with greatly diminished fat pads. Immunohistological examination of the pancreata showed a marked decrease in islet insulin content and marked CD4<sup>+</sup> lymphocytic proliferation, but not CD8<sup>+</sup> proliferation, both around and within the islets (Fig. 5), which was not seen in the controls (data not shown). Although no similar study has been

reported for PARP-deficient mice, rodents pretreated with 3-ab developed  $\beta$ -cell specific insulomas 1 year after receiving a single high dose of STZ (36, 40). Thus, the method of cell death reported here, resulting from the persistence of DNA adducts or the lack of DNA strand breaks, may have implications for the generation of secondary tumors following treatment with chemotherapeutic alkylating agents.

**Effect of APNG deletion on MLDS-induced  $\beta$ -cell apoptosis.** Since MLDS is a commonly used rodent model of type 1 diabetes, APNG<sup>+/+</sup> and APNG<sup>-/-</sup> mice were treated with STZ (40 mg/kg) for 5 days, and 6 h after the last dose their pancreata were dissected for histological examination. Pancreata were also removed on day 11 to determine the effect of this regimen on pancreatic insulin levels in these mouse strains. On day 5, islets from both strains showed evidence of apoptosis, with APNG<sup>-/-</sup> mice showing a 2.5-fold increase in  $\beta$ -cell apoptosis compared with their normal littermates (Table 3). By day 11, however, this situation had been reversed (Table 3), with less apoptosis being present in APNG<sup>-/-</sup> mice. At this later time, pancreatic insulin levels were approximately 30% of control values in both mouse strains (data not shown), and this was borne out by immunohistochemical staining for insulin, which showed a decrease in staining intensity for both STZ-treated APNG-deficient and normal mice (Fig. 6A to D). Islets from both strains showed evidence of a low-grade CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytic infiltration at this time (Fig. 6E to J). Therefore, under this STZ regimen, both mouse strains succumbed similarly to diabetes. However, evidence from the cell morphology studies suggests that the initial signaling events leading to apoptosis are different and occur more rapidly in the absence of APNG.

## DISCUSSION

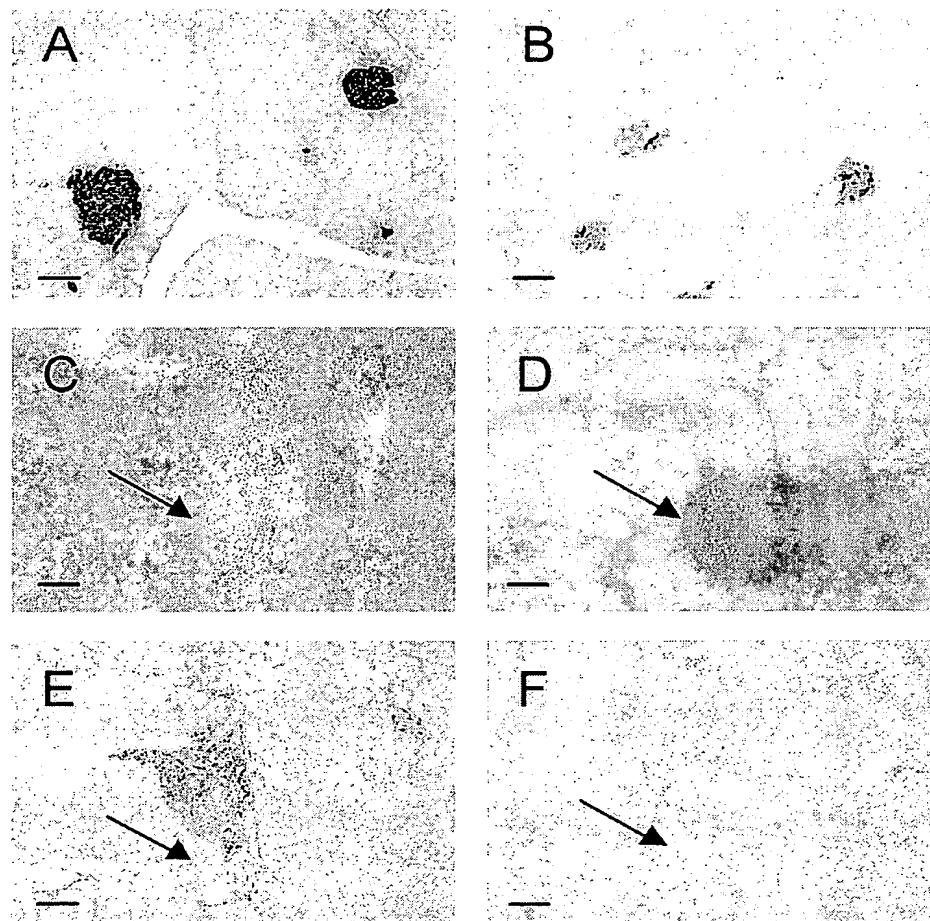
This study demonstrates that APNG<sup>-/-</sup> mice are essentially resistant to the immediate cytotoxic effects of a single high dose of STZ, analogous to that described for PARP-deficient mice (2, 25, 33). However, significant differences in the extent and timing of islet cell apoptosis were observed in both this and the MLDS model of type 1 diabetes, suggesting that at least a subset of the unrepaired DNA adducts can act as signals for apoptosis. The finding that both APNG<sup>-/-</sup> and PARP-deficient mice are resistant to single-high-STZ-dose-induced  $\beta$ -cell necrosis provides further evidence that the necrosis is caused by DNA adduct removal, the subsequent induction of DNA strand breaks by AP-endonuclease, and the activation of PARP. This is also supported by our observation that in contrast to APNG<sup>+/+</sup> islets, APNG<sup>-/-</sup> islets treated in vitro do not show significant PARP activation. Since APNG<sup>-/-</sup> and APNG<sup>+/+</sup> mice have the same basal levels of PARP activity, the finding

TABLE 2. Long-term effects of a single high dose of STZ on blood glucose and pancreatic insulin levels in APNG<sup>-/-</sup> mice

Treatment	Blood glucose level (mM) <sup>a</sup> (no. of mice)	Pancreatic insulin level (ng/mg) <sup>a</sup> (no. of mice)
None (controls)	5.48 ± 0.89 (7)	82.3 ± 28 (6)
STZ (140 mg/kg)	13.1 ± 3.3 (11) <sup>b</sup>	7.97 ± 5.19 (7)

<sup>a</sup>  $P < 0.001$  for comparisons between controls and STZ-treated mice. Values are expressed as the mean ± standard deviation.

<sup>b</sup> Values do not include one animal, where the glucose level was >44 mM.



**FIG. 5. Immunohistochemical determination of autoimmune diabetes in APNG<sup>-/-</sup> mice 8 months after treatment with STZ.** (A and B) Formalin-fixed sections from untreated (A) and STZ-treated (B) APNG<sup>-/-</sup> mice were stained for insulin as described in Materials and Methods. (C to F) CD4<sup>+</sup> and CD8<sup>+</sup> immunostaining was carried out on frozen sections. Hematoxylin-eosin (C) and insulin (D) staining indicate the position of the islet in the section, while staining for the specific lymphocyte markers shows evidence of CD4<sup>+</sup> (E) but not CD8<sup>+</sup> (F) lymphocytic invasion in the islet. The more porous nature of the frozen sections compared to the formalin-fixed sections made them unsuitable for quantitative assessment of insulin content by this method. Arrows indicate the position of the islet in the section. Bar, 100  $\mu$ m.

that APNG<sup>-/-</sup> mice lack a significant STZ-induced activation of PARP indicates that the majority of STZ-induced DNA strand breaks are due to the action of APNG on DNA adducts that are substrates for this enzyme.

The presence of significant amounts of STZ-induced  $\beta$ -cell apoptosis in APNG<sup>-/-</sup> mice is a novel finding and supports previous reports that PARP inhibitors protect cultured  $\beta$  cells against STZ-induced necrosis but not against cytokine-induced apoptosis (16, 18). To our knowledge, this is the first *in vivo* evidence of increased apoptosis resulting from a reduced repair of DNA adducts and supports a previous report of the induction of apoptosis in cell lines lacking APNG treated *in vitro* with MeOSO<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>-lexitropsin, which almost exclusively forms 3-meA adducts (10). 3-meA is known to block DNA replication by inhibiting the action of DNA polymerases (23), and thus it is likely that stalled replication-transcription forks at 3-meA adducts are the ultimate apoptotic signaling lesions in STZ-treated APNG<sup>-/-</sup> cells. Since 3-meA in DNA

has a half-life of only 24 h under physiological conditions *in vitro* (24), its potential toxicity in cells undergoing replication decreases with time, and this probably explains the timing of the peak of apoptosis observed in islets from APNG<sup>-/-</sup> mice after STZ treatment. However, this could also be due in part to

**TABLE 3. Islet cell apoptosis following MLDS treatment of APNG<sup>+/+</sup> and APNG<sup>-/-</sup> mice**

Mouse strain	Day	% Apoptosis (no. of mice) <sup>a</sup>
APNG <sup>+/+</sup>	5	0.98 $\pm$ 0.1 <sup>b</sup> (5)
	11	1.56 $\pm$ 0.12 <sup>c</sup> (11)
APNG <sup>-/-</sup>	5	2.42 $\pm$ 0.21 <sup>b</sup> (5)
	11	1.03 $\pm$ 0.18 <sup>c</sup> (11)

<sup>a</sup> Values are expressed as the mean  $\pm$  the standard error of the mean.

<sup>b</sup> Day 5 values ( $P < 0.001$ ).

<sup>c</sup> Day 11 values ( $P < 0.05$ ).

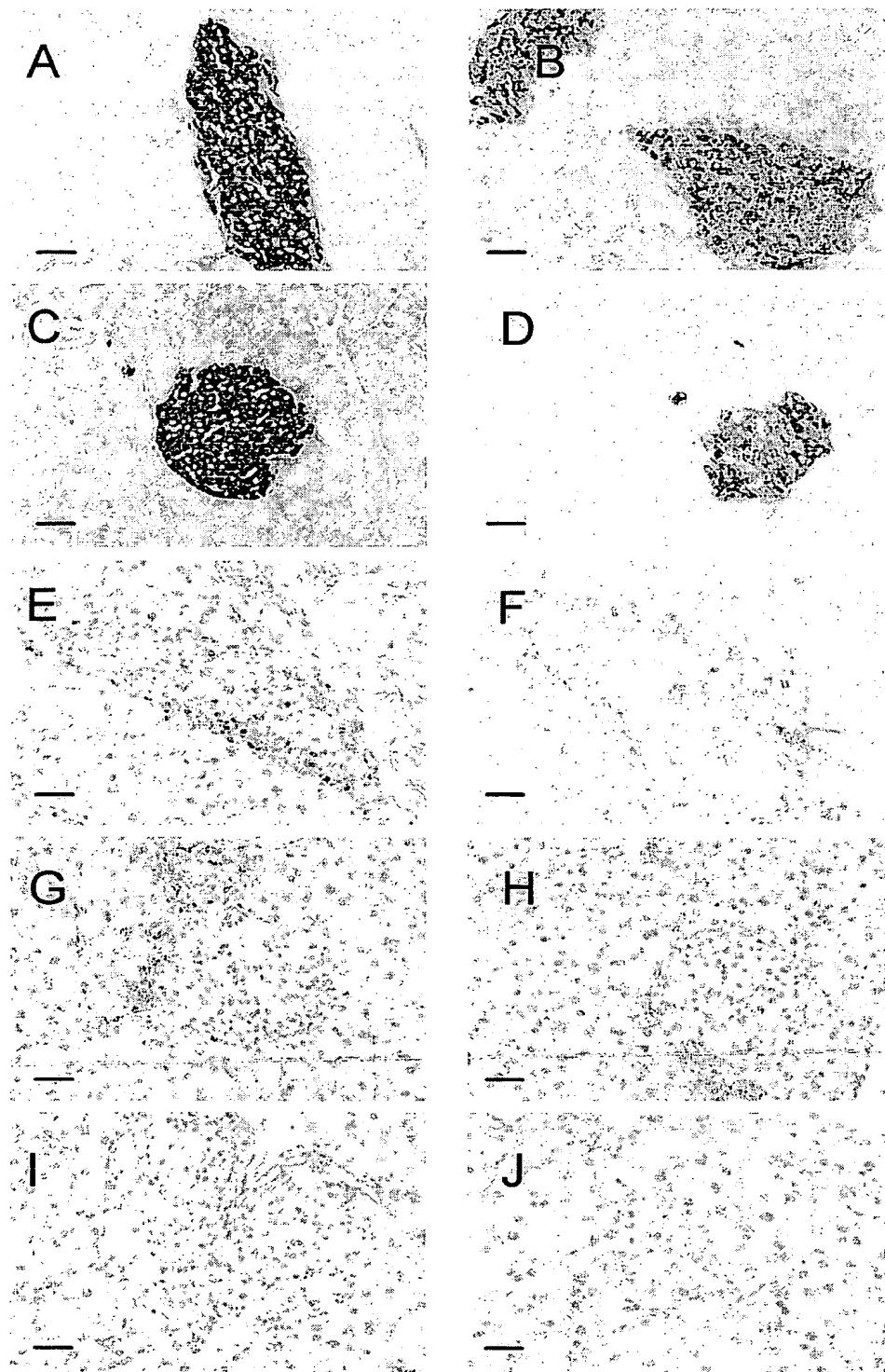


FIG. 6. Immunohistochemical determination of MLDS-induced autoimmune diabetes in APNG<sup>+/+</sup> and APNG<sup>-/-</sup> mice on day 11. (A to D) Formalin-fixed pancreata stained for insulin. (E to J) Frozen sections of pancreata stained for CD4<sup>+</sup> (E, G, and I) or CD8<sup>+</sup> (F, H, and J). (A and B) APNG<sup>+/+</sup> control and MLDS, respectively; (C and D) APNG<sup>-/-</sup> control and MLDS, respectively; (E and F) APNG<sup>+/+</sup>; (G and H) APNG<sup>-/-</sup>; (I and J) control sections from untreated APNG<sup>-/-</sup> mice. Bar, 50  $\mu$ m.

the formation of DNA strand breaks resulting from the spontaneous depurination of 3-meA over this period.

Evidence that 3-meA depurination was not the major apoptotic signaling event was obtained from studies of islet cell morphology following STZ treatment: 3-ab-pretreated APNG<sup>+/+</sup> mice showed significantly less  $\beta$ -cell apoptosis than did APNG<sup>-/-</sup> mice, consistent with the hypothesis that the unrepaired DNA adducts, or a fraction thereof, act as the apoptotic signal. However, the degree of cell death, as measured by pancreatic insulin levels, did not differ between APNG<sup>-/-</sup> and PARP-inhibited APNG<sup>+/+</sup> mice. Considering the kinetics of apoptotic cell death over time, the degree of apoptosis seen in APNG<sup>-/-</sup> mice would adequately explain the fall in pancreatic insulin levels in these mice. Since the mode of STZ-induced  $\beta$ -cell death in 3-ab-pretreated APNG<sup>+/+</sup> mice is unclear, it is possible that there may be another mechanism of delayed  $\beta$ -cell death. STZ is known to damage mitochondria, inhibiting ATP production (7), and this would lead to loss of membrane integrity and necrosis. Necrosis by this mechanism, therefore, may not involve nuclear DNA damage detectable as pyknotic nuclei 8 h postdose and may be difficult to detect by light microscopy, especially if it is a minor pathway.

Apoptosis is the mode of  $\beta$ -cell death in the NOD mouse model (28). DNA adduct formation and APNG may also play a role in the NOD mouse model and in immune-mediated  $\beta$ -cell apoptosis. In addition to the *N*-methylpurines, which are an integral part of methylation damage, APNG releases 1,N<sup>6</sup>-ethenoadenine and deaminated adenine, both of which are generated endogenously by a number of processes, including macrophage-induced NO synthesis and lipid peroxidation (9, 11, 26). Cytokine-generated reactive oxygen species cause DNA strand breaks and  $\beta$ -cell apoptosis in vitro and are thought to play a role in the induction of the immune-mediated  $\beta$ -cell apoptosis seen in NOD mice (35). Thus, it is possible that APNG may also protect against NO and some immune-mediated apoptosis. Consistent with the idea that low levels of APNG may contribute to susceptibility to autoimmunity in NOD mice is the finding that NOD mice are very sensitive to multiple low doses of STZ but relatively resistant to a single high dose of STZ (32). The present study suggests that strain differences in sensitivity to STZ could be explained by differences in APNG activity and thus supports a recent article reporting differences in DNA strand break induction and PARP activation in two mouse strains (4).

The finding that APNG<sup>-/-</sup> mice treated with a single high dose of STZ develop a delayed lymphocytic proliferation and diabetes contrasts with earlier reports on the induction of  $\beta$ -cell tumors in rats given combined treatments of STZ and PARP inhibitors (36, 40). These are important results since they indicate that (i) inhibition of PARP may contribute to carcinogenesis and (ii) the persistence of DNA adducts can lead to an autoimmune reaction. It is possible that other adducts such as O<sup>6</sup>-methylguanine or DNA lesions induced by oxidative damage are responsible for the tumors seen in PARP-inhibited mice. Alternatively, the fidelity of DNA repair synthesis may be compromised in PARP-inhibited  $\beta$  cells since strand rejoining is known to occur more slowly in PARP-treated cells (39). On the other hand, while the deletion of APNG greatly reduces the ability of the cells to carry out base excision repair of these adducts, perhaps crucially, the signal-

ing apparatus for other types of DNA damage, such as that caused by reactive oxygen species, remains in place. Relevant to this is the recent finding that PARP can promote inflammation through its interaction with the redox-regulated transcription factor NF- $\kappa$ B (31). In response to many agents, including genotoxins and oxidative stress, this family of transcription factors is involved both in the up-regulation of expression of inducible nitric oxide synthase and several proinflammatory cytokines and in the prevention of apoptosis initiation (13). Indeed, the inappropriate expression of NF- $\kappa$ B and the resulting autoimmune and inflammatory response has been proposed as the critical event in the development of type 1 diabetes (17). Thus, in APNG<sup>-/-</sup> mice the biological consequence of an active NF- $\kappa$ B signaling system is susceptibility to STZ-induced autoimmune diabetes. We are currently assessing the biological response of PARP<sup>-/-</sup> and APNG-PARP double-null mice in this system.

The lack of both  $\beta$ -cell apoptosis and any reported autoimmunity in PARP-inhibited mice treated with a single high dose of STZ suggests that  $\beta$ -cell apoptosis and not necrosis may be necessary for the induction of the autoimmune reaction. These findings are in keeping with recent studies by O'Brien et al., who showed that PARP-inhibited NOD mice are protected from both  $\beta$ -cell apoptosis and the ensuing autoimmune reaction (29). The observation that the immune reaction is CD4 positive suggests a role for antigen-presenting cells in the activation of the immune system. It is possible, then, that the difference between apoptotic and necrotic stimulation of the immune system may lie in the extent to which antigen-presenting cells are activated after taking up the remains of the  $\beta$  cell.

In the MLDS model, the timing and extent of apoptosis in APNG<sup>-/-</sup> mice is consistent with a role for unrepaired 3-meA adducts in this pathway. Although a proportion of the DNA adducts can be removed in normal mice, daily treatment of STZ would lead to an increase in the residual number of unrepaired adducts. Thus, APNG<sup>-/-</sup> mice may show more apoptosis than APNG<sup>+/+</sup> mice by virtue of their inability to remove STZ-induced DNA adducts. This is further supported by the observation that APNG<sup>-/-</sup> mice treated with a single high dose of STZ also show  $\beta$ -cell apoptosis. For MLDS, the cumulative effect of the STZ treatment resulted in the same biological outcome, irrespective of APNG status. A major unresolved issue is why the autoimmune reaction is delayed 8 months after a single high dose of STZ in APNG<sup>-/-</sup> mice but occurs at day 11 in the MLDS model. One explanation may be that a single high dose of STZ may not produce the same T-lymphocyte imbalance that the MLDS regimen is thought to produce. Additionally, as C57BL/6J mice age, they develop immune dysregulation, again involving NF- $\kappa$ B signaling (34). Thus, autoimmunity may require both apoptotic  $\beta$ -cell priming of antigen-presenting cells and dysregulation of the lymphocyte subsets. Further studies are required to determine the relationship between the persistence of DNA adducts, apoptosis, and the immune response.

In conclusion, this study has revealed three important findings: (i) APNG deficiency leads to resistance to STZ-induced necrosis, (ii) DNA adduct persistence can lead to cellular apoptosis in vivo, and (iii) the inhibition of base excision repair before the induction of DNA strand breaks can radically alter

the biological outcome, preventing the onset of tumorigenesis and promoting autoimmunity.

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## Protein kinase C and the sub-sensitivity and sub-reactivity of the diabetic rat prostate gland to noradrenaline

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### Abstract

Concentration-response curves to noradrenaline (1 nM–100 μM) were obtained in prostates from 6-week streptozotocin diabetic, insulin-treated diabetic or control rats. Compared to the curve obtained in controls, those obtained in prostates from diabetic and insulin-treated diabetic rats were shifted rightward. The  $\alpha_1$ -adrenoceptor antagonist, prazosin (100 nM), caused a rightward shift of the curves in prostates from all groups. In contrast, the uptake 1 inhibitor, nisoxetine (300 nM), only produced a leftward shift of the curves in prostates from control and insulin-treated diabetic rats. However, frequency-response curves obtained in prostates from both control and diabetic rats were shifted leftward by nisoxetine (300 nM). The concentration-response curve to the  $\alpha_1$ -adrenoceptor agonist, methoxamine (10 nM–100 μM), obtained in prostates from diabetic rats was shifted rightward compared with controls. Calphostin C (500 nM), a protein kinase C inhibitor, caused a leftward shift of the curve in prostates from diabetic, but not control, rats. The protein kinase C inhibitor, bisindolylmaleimide I (500 nM),  $\beta$ -adrenoceptor antagonist, propranolol (500 nM) and muscarinic cholinoreceptor antagonist, atropine (300 nM), had no effect on the noradrenaline concentration-response curves of prostates from control or diabetic rats. Our results suggest that diabetes reduces the sensitivity and reactivity of the prostate to noradrenaline-induced stimulation, and this reduction may be due to changes in protein kinase C activity. © 2002 Elsevier Science B.V. All rights reserved.

**Keywords:** Diabetes mellitus, type I; Prostate gland; Benign prostatic hyperplasia; Noradrenaline; Protein kinase C

### 1. Introduction

The prostate gland is a major accessory gland of the male reproductive system and plays an important role in maintaining the viability of sperm and assisting its passage through the female reproductive tract. It is a tubuloalveolar gland consisting of two lobes contained within a capsule. The gland is composed of secretory alveoli surrounded by fibro-elastic connective tissue interspersed with smooth muscle cells (Crowe et al., 1987). The rat prostate gland, like that of the human, is located around the urethra, in close

proximity to where the urethra joins the bladder. Both the sympathetic and parasympathetic nervous systems innervate the rat prostate gland (McVary et al., 1998), but contractile responses to electrical nerve stimulation are mediated by  $\alpha_1$ -adrenoceptors (Lau et al., 1998; Nishi et al., 1998).

Benign prostatic hyperplasia is the nonmalignant growth of the prostate gland and, due to the strategic position of the prostate, frequently produces problematic lower urinary tract symptoms in older men (Madsen and Bruskewitz, 1995). It is produced by an age- and androgen-dependent increase in the physical size of the gland, as well as an increase in the sympathetic tone of the prostatic smooth muscle, which consequently puts added pressure on the urethra (Cooper et al., 1999). The predominant pharmacological treatment of benign prostatic hyperplasia involves  $\alpha_1$ -adrenoceptor antagonists, such as prazosin (Hedlund and Andersson, 1988) and terazosin (Lepor et al., 1996), which act by decreasing the sympathetic stimulation of the prostatic smooth muscle. These drugs are used to reduce problematic symptoms and to improve urinary flow (Cooper et al., 1999).

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Recent epidemiological studies have shown a correlation between diabetes and the symptoms of benign prostatic hyperplasia in patients without an enlarged prostate (Klein et al., 1999). This suggests that diabetes is related to the increased sympathetic tone component of benign prostatic hyperplasia. Another study found a correlation between the development of benign prostatic hyperplasia and men with Type II, non-insulin-dependent diabetes mellitus, obesity, and/or hyperinsulinaemia indicating that these may all be risk factors (Hammarsten and Hogstedt, 1999). Based on evidence that both obesity (Troisi et al., 1991) and hyperinsulinaemia (Rowe et al., 1981) are conditions associated with an increase in sympathetic outflow, it was also suggested that men with fast-growing benign prostatic hyperplasia might have increased sympathetic nerve activity. Furthermore, this effect may be due to the sympatho-excitatory effect of insulin (Rowe et al., 1981).

Previous studies have shown that streptozotocin-diabetic rats display a significant reduction in body weight (James and Hodgson, 1997) and serum insulin level, as well as high serum glucose levels (James and Hodgson, 1997), and decreases in fertility and spermatogenesis (Frenkel et al., 1978) when compared to nondiabetic rats. They also exhibit parallel reductions in reproductive organ weights, including the prostate gland (Crowe et al., 1987; Nishi et al., 1998). This reduction in body and prostatic weight can be prevented or reversed by early chronic insulin administration (Crowe et al., 1987; Nishi et al., 1998).

Induction of diabetes mellitus causes a significant decrease in catecholamine-containing nerve fibres of the rat prostate (Crowe et al., 1987), as well as large reductions in the densities of prostatic  $\alpha_1$ - (Crowe et al., 1987) and  $\beta$ -adrenoceptors (Gousse et al., 1991). Streptozotocin-diabetic rats also display decreased densities of prostatic muscarinic receptors (Latifpour et al., 1991), an effect which is prevented or reversed with insulin treatment (Fukurnoto et al., 1993).

Protein kinase C has been shown to play a role in  $\alpha_1$ -adrenoceptor-mediated contraction of guinea-pig vas deferens (Kamimura et al., 2000). Protein kinase C in the rat ventral prostate is of the  $\text{Ca}^{2+}$ -dependent form, and is thought to play an important role in the physiological activities and mechanisms of cell proliferation and differentiation in the prostate gland (Garcia-Paramio et al., 1993). Prostate glands from streptozotocin-diabetic rats have also been shown to exhibit an increased protein kinase C activity in the membrane fraction, and a decreased protein kinase C activity in the cytosolic fraction, when compared with control rats (Garcia-Paramio et al., 1995). This effect was found to be restored toward control conditions by insulin treatment (Garcia-Paramio et al., 1993).

Although it is widely accepted that enlargement of the prostate gland is inevitable as men age, the mechanisms involved in the development of benign prostatic hyperplasia are largely unknown. To date, investigations into the diabetes-induced alterations in prostatic receptors and prostate size have only been preliminary. This study investigated whether

changes occur in the sensitivity and reactivity to noradrenaline of prostate glands from diabetic rats in addition to changes in size.

## 2. Materials and methods

### 2.1. Animals

Male Wistar rats were weighed and lightly anaesthetised (4% halothane, 2:1  $\text{O}_2/\text{N}_2\text{O}$ ) to enable measurement of blood glucose levels via a tail vein sample. Blood glucose was measured using an Ames Glucometer II. Diabetes was induced by a single tail vein injection of streptozotocin (60 mg/kg), dissolved immediately prior to use in citrate buffer (50 mM citric acid and 50 mM trisodium citrate; pH 4.5). An equivalent volume of citrate buffer was injected into age-matched control rats. Rats were housed in treatment pairs (one diabetic and one control) for a 6-week period.

A subgroup of rats was treated with a single daily dose of Lente insulin (4 units for the first 3 days, and 6 units every day thereafter, s.c.; Monotard human insulin zinc suspension) commencing 2 days after streptozotocin administration. Ethical approval for all experiments was obtained from the Monash University Pharmacology Animal Ethics Committee.

### 2.2. Isolated organ bath experiments

After a 6-week period, rats were weighed, killed and blood glucose was measured. A lower abdominal incision was made and prostate glands were dissected out and vertically mounted onto wire tissue holders in 5-ml organ baths. Experiments were paired with one prostate lobe placed in Krebs solution ((mM): NaCl 118.4, KCl 4.7,  $\text{NaHCO}_3$  2.5,  $\text{KH}_2\text{PO}_4$  1.2,  $\text{MgSO}_4$  1.2,  $\text{CaCl}_2$  2.5, and glucose 11.1) and the other placed in Krebs solution containing an antagonist or inhibitor, as indicated. Following a 1-h equilibration period, discrete dose-response curves to noradrenaline (1 nM–100  $\mu\text{M}$ ) or methoxamine (10 nM–100  $\mu\text{M}$ ) were performed. Agonists were kept in contact with the tissue until the response plateaued (approximately 10–15 s), with a 10-min period between each dose.

In a subset of experiments, isolated prostate preparations were electrically field stimulated. Tissues were mounted onto perspex tissue holders incorporating platinum electrodes connected to a Grass S88 stimulator. Parameters for stimulation applied to tissues were: 10 pulse trains of 0.5-ms duration, at 80 V. The frequencies tested were 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10 and 20 Hz. Stimulation at frequencies greater than 1 Hz were given as 10-s trains. A resting period of 10 min was allowed between stimulations.

### 2.3. Histochemical studies

Rats from all three treatment groups (see Section 2.1) were killed and prostate glands removed. Tissues were placed in

Tissue TEK and frozen at  $-21^{\circ}\text{C}$  to be stained for norepinephrine. At least 12 sections ( $12 \mu\text{m}$ ) were cut from each prostate specimen and thawed onto gelatin-coated slides. Prostates were exposed to sucrose–potassium phosphate–glyoxylic acid solution (SPG) for 3 s (De la Torre and Surgeon, 1976). Sections were dried for approximately 20 min and heated at  $80^{\circ}\text{C}$  for an additional 5 min. Slices were mounted in paraffin oil and examined with an Olympus photomicroscope fitted with an Olympus mercury burner fluorescent light source attachment and DM55 dichroic mirror, BP400–410 exciter filter and BA 455 barrier filter.

For staining with haematoxylin and eosin, tissues were fixed for 2 h in a solution containing 4% paraformaldehyde in phosphate buffered saline (PBS; (mol/l) NaCl 0.137,  $\text{KH}_2\text{PO}_4$  0.002 and  $\text{Na}_2\text{HPO}_4$  0.008). Tissues were washed four times, each for 10 min, in a solution containing 7% sucrose and 0.01% sodium azide in PBS. Prostates were stored in this solution for 48 h at  $4^{\circ}\text{C}$ . Tissues were placed in Tissue TEK, snap frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ . At least 12 sections ( $12 \mu\text{m}$ ) were cut from each prostate specimen and thawed onto gelatin-coated slides. Sections were stained routinely in Mayer's Haemalum for 10 min, rinsed in distilled water for 2 min, and placed in Scott's solution for a further 2 min. After rinsing in gentle running tap water for 2 min, prostate slices were stained in Eosin for 30 s and carefully rinsed again in tap water for 3 min. Sections were dehydrated once in 70% and 90% ethanol for 4 min, and three times in 100% ethanol for 4 min each. Histoclear was used, three times for 4 min each, to clear sections, and slides were mounted in DPX and coverslipped before viewing under an Olympus BX60 microscope. Micrographs were taken using an Olympus PM 30 photographic system.

#### 2.4. Drugs and solutions

Drugs used included: the muscarinic receptor antagonist, atropine sulfate (Sigma); the protein kinase C inhibitor, bisindolylmaleimide I (Calbiochem); protein kinase C inhibitor, calphostin C (Calbiochem);  $\alpha_1$ -adrenoceptor agonist,

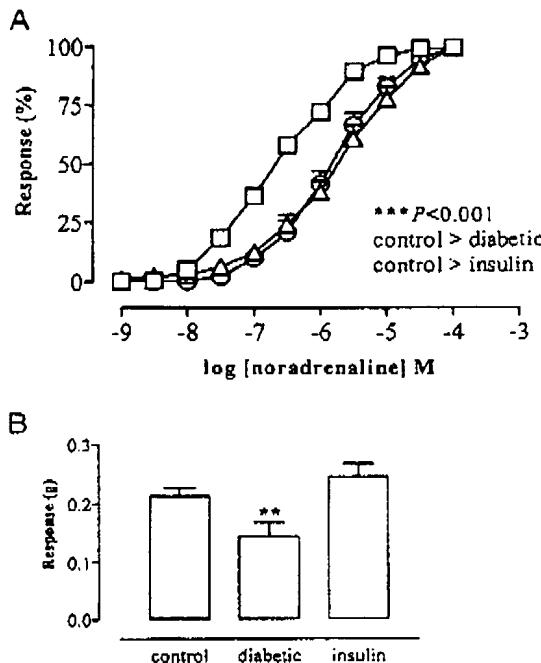


Fig. 1. (A) Discrete dose-response curves of prostates from control (□,  $n=41$ ), diabetic (△,  $n=36$ ) and insulin-treated diabetic (○,  $n=14$ ) rats to norepinephrine. Data are expressed as means  $\pm$  S.E.M. (B) The maximum response of prostate glands from control ( $n=41$ ), diabetic ( $n=36$ ) and insulin-treated ( $n=14$ ) diabetic rats to norepinephrine. Data are expressed as means  $\pm$  S.E.M. \*\*  $P < 0.01$ , when compared with the control group.

methoxamine hydrochloride (Wellcome); uptake 1 inhibitor, nisoxetine hydrochloride (Eli Lilly);  $\alpha$ -adrenoceptor agonist, noradrenaline bitartrate (Sigma);  $\alpha$ -adrenoceptor antagonist, prazosin hydrochloride (Sigma);  $\beta$ -adrenoceptor antagonist, propranolol hydrochloride (ICI).

Atropine, nisoxetine, prazosin, and propranolol were all dissolved in distilled water. Calphostin C and bisindolylmaleimide I were dissolved in 1% dimethyl sulphoxide. Noradrenaline and methoxamine were dissolved in a catecholamine diluent (0.9% NaCl, 0.0156%  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ , 0.004% ascorbic acid). All subsequent dilutions were made in distilled water.

#### 2.5. Statistical analysis

The data are expressed as a percentage of the maximum response achieved by each individual tissue, and are presented as means  $\pm$  S.E.M. Differences in mean concentration-response curves of two or more treatment groups were compared at all concentrations on the log concentration-response curve using a two-way repeated measure analysis of variance (ANOVA). Differences in maximum response, blood glucose levels and body and prostate weights between treatment groups were compared using a one-way ANOVA. Bonferroni correction for multiple comparisons was per-

Table 1  
Body weights, blood glucose levels and prostate weights of control ( $n=41$ ), diabetic ( $n=36$ ) and insulin-treated diabetic ( $n=14$ ) rats

	Body weight (g)		Blood glucose (mM)		Prostate weight (mg)
	Initial	Final	Initial	Final	
Control	$329 \pm 4$	$451 \pm 5^a$	$7.1 \pm 0.2$	$7.6 \pm 0.2$	$93.3 \pm 5.2$
Diabetic	$329 \pm 3$	$282 \pm 4^a$	$7.1 \pm 0.2$	$21.4 \pm 0.5^b$	$41.8 \pm 6.5^c$
Insulin	$328 \pm 4$	$390 \pm 5^a$	$7.2 \pm 0.4$	$6.3 \pm 1.5$	$38.3 \pm 3.0^d$

Initial measurements were made at the time of streptozotocin or vehicle injection, and final measurements made 6 weeks later.

<sup>a</sup> Significantly different from initial value for corresponding treatment group,  $P < 0.01$ .

<sup>b</sup> Significantly different from initial value for corresponding treatment group,  $P < 0.001$ .

<sup>c</sup> Significantly different from corresponding values from control group,  $P < 0.001$ .

formed when required. In all cases,  $P \leq 0.05$  was taken as statistically significant.

$EC_{50}$  values were determined using Graph Pad Prism (version 2.0), and were used to calculate 95% confidence intervals and the shift of the noradrenaline concentration-response curves in the presence of an antagonist or inhibitor from its corresponding control.

### 3. Results

#### 3.1. Laboratory animals

The mean body weights of 6-week control (vehicle-treated) and insulin-treated diabetic rats were significantly increased compared to their corresponding mean pre-injection

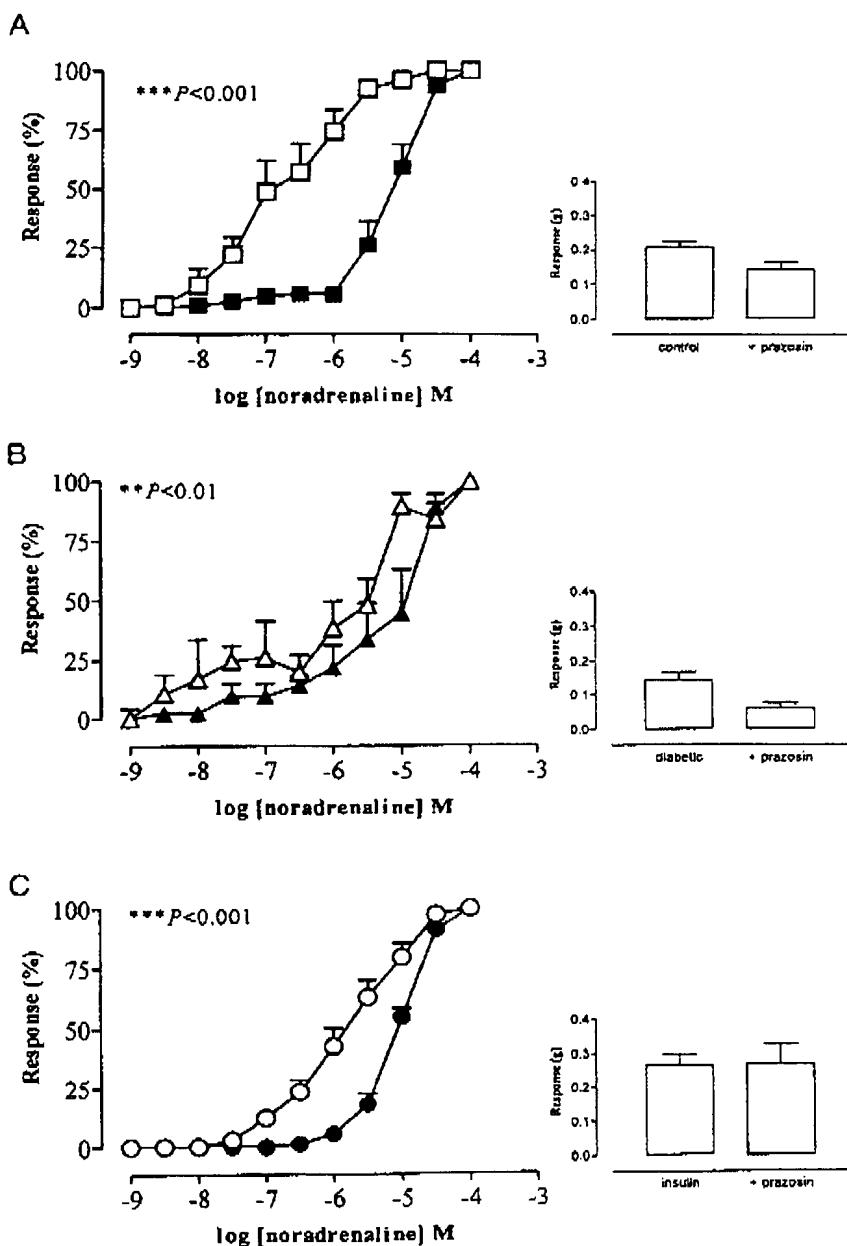


Fig. 2. The effect of prazosin (100 nM) on the noradrenaline concentration-response curve of prostate glands from (A) control ( $\square$ ,  $n = 7$ ), (B) diabetic ( $\Delta$ ,  $n = 6$ ) and (C) insulin-treated diabetic ( $\circ$ ,  $n = 6$ ) rats. Solid symbols represent response in the presence of prazosin. Histogram: maximum response of tissues in the absence and presence of prazosin. Data are expressed as means  $\pm$  S.E.M. \*\* $P < 0.01$  and \*\*\* $P < 0.001$ , compared with response in the absence of prazosin.

weights ( $P < 0.01$ ). In contrast, diabetic rats displayed significantly reduced mean body weights when compared with their mean pre-injection weights ( $P < 0.01$ ) (Table 1).

Mean blood glucose levels of control and insulin-treated diabetic rats remained normoglycaemic. However, diabetic rats exhibited significantly increased mean blood glucose

levels compared to their corresponding pre-injection levels ( $P < 0.001$ ; Table 1).

Mean wet weights of prostate tissue from diabetic and insulin-treated diabetic rats were significantly reduced when compared to prostate glands from age-matched control rats ( $P < 0.001$ ) (Table 1).

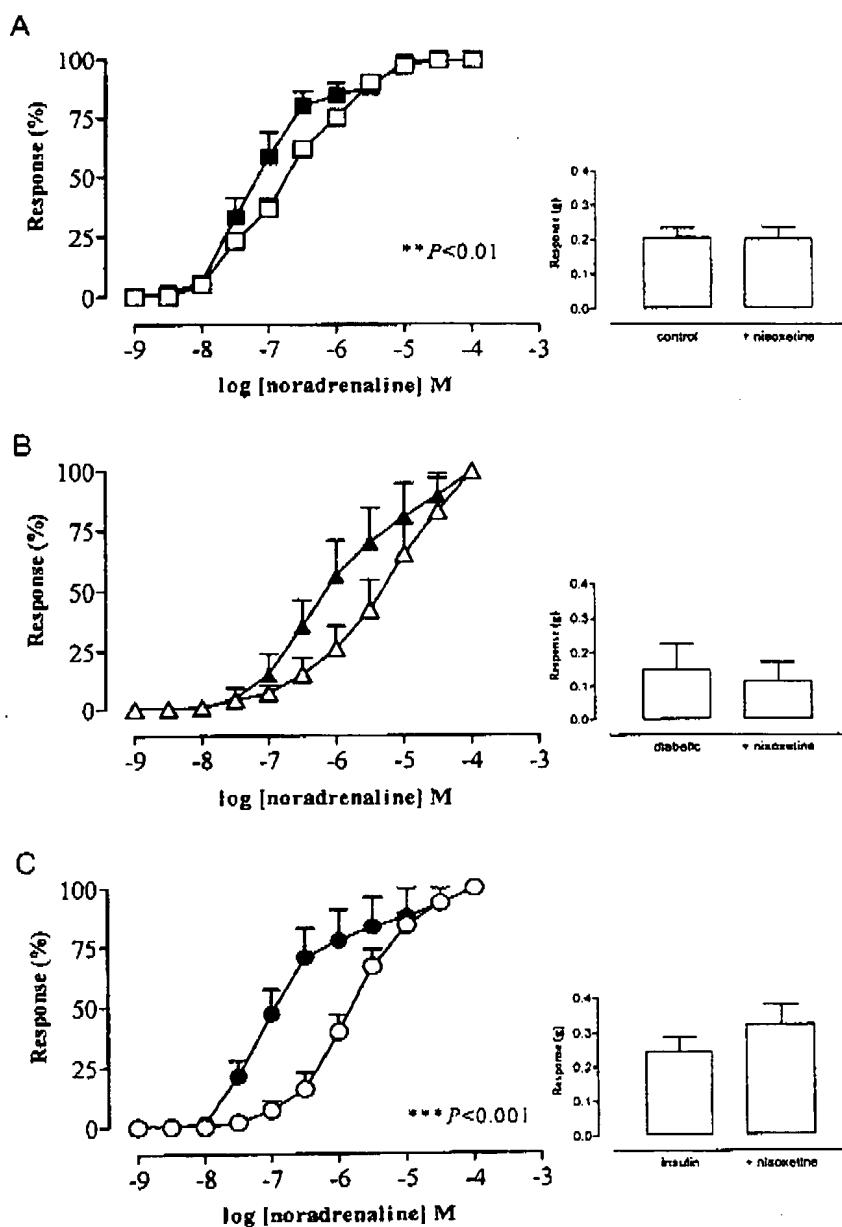


Fig. 3. Effect of nisoxetine (300 nM) on the noradrenaline concentration-response curve of prostate glands from (A) control ( $\square$ ,  $n = 9$ ), (B) diabetic ( $\Delta$ ,  $n = 7$ ) and (C) insulin-treated diabetic ( $\circ$ ,  $n = 6$ ) rats. Solid symbols represent response in the presence of nisoxetine. Histogram: maximum response of tissues in the absence and presence of nisoxetine. Data are expressed as means  $\pm$  S.E.M. \*\*  $P < 0.01$  and \*\*\*  $P < 0.001$ , compared with corresponding curve in the absence of nisoxetine.

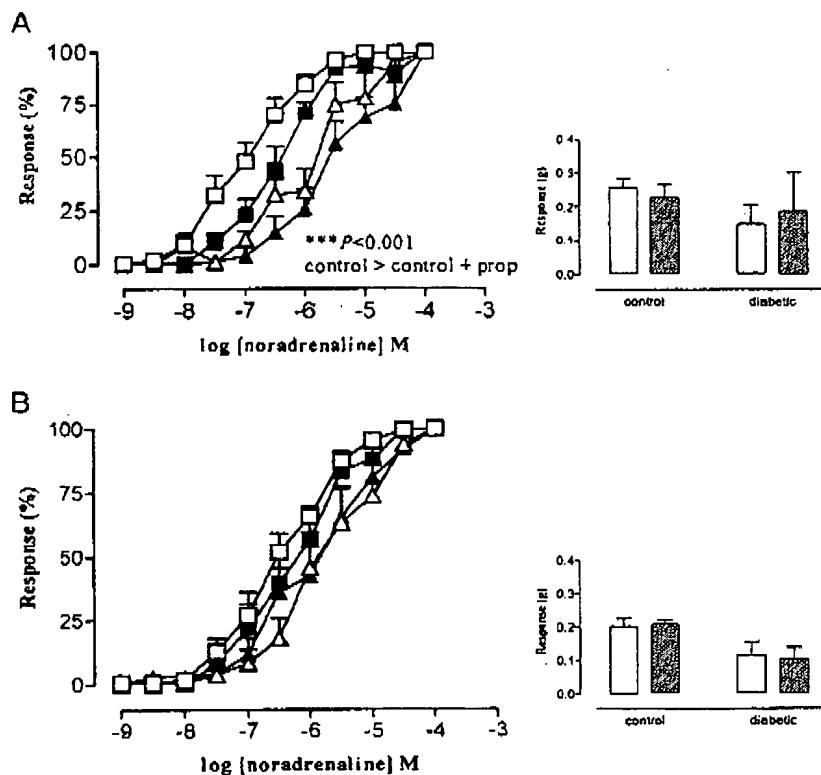


Fig. 4. (A) Effect of propranolol (1 μM) on the noradrenaline concentration–response curve of prostate glands from control (□,  $n=6$ ) and diabetic (△,  $n=6$ ) rats. Solid symbols represent response in the presence of propranolol. Histogram: maximum response of tissues in the absence (open bars) and presence (cross hatched bars) of propranolol. Data are expressed as means ± S.E.M. (B) Effect of atropine (300 nM) on the noradrenaline concentration–response curve of prostate glands from control (□,  $n=7$ ) and diabetic (△,  $n=6$ ) rats. Solid symbols represent response in the presence of atropine. Histogram: maximum response of tissues in the absence (open bars) and presence (cross hatched bars) of atropine. Data are expressed as means ± S.E.M.

### 3.2. Effects of noradrenaline

Discrete addition of noradrenaline (1 nM–100 μM) produced dose-dependent contractions in prostates from all rats. The mean noradrenaline concentration–response curve ob-

tained in tissues from diabetic and insulin-treated diabetic rats were shifted rightward 8.5-fold (95% confidence limit = 4.1–17.4) and 6.7-fold (95% confidence limit = 3.9–11.5), respectively, compared to the mean noradrenaline concentration–response curve obtained in tissues from con-

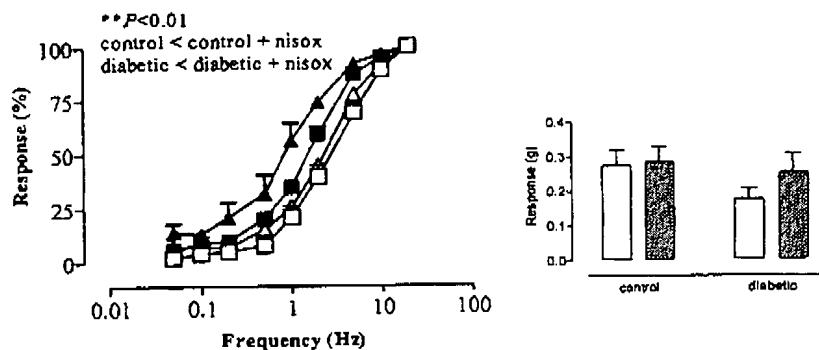


Fig. 5. The effect of nisoxetine (300 nM) on the frequency response curve of electrically field stimulated prostates from control (□,  $n=6$ ) and diabetic (△,  $n=5$ ) rats. Solid symbols represent response in the presence of nisoxetine. EFS parameters: 10 trains of 0.5-ms duration; 80 V; frequency 0.05–20 Hz every 10 min. Histogram: maximum response of tissues in the absence (open bars) and presence (cross hatched bars) of nisoxetine. Data are expressed as means ± S.E.M.

trol rats ( $P < 0.001$ ) (Fig. 1A). The noradrenaline concentration-response curve obtained in preparations from insulin-treated diabetic rats did not differ from that seen in tissues from diabetic rats (Fig. 1A). The mean maximum response to noradrenaline in prostate glands from diabetic rats was significantly decreased when compared with the maximum responses observed in tissues from control and insulin-treated diabetic rats ( $P < 0.01$ ) (Fig. 1B).

### 3.3. Effects of the $\alpha$ -adrenoceptor antagonist, prazosin

Prazosin (100 nM) produced significant 60.1-fold (95% confidence limit = 24.4–147.9;  $P < 0.001$ ) and 3.8-fold (95% confidence limit = 0.7–19.7;  $P < 0.01$ ) rightward shifts of the mean noradrenaline concentration-response curves in tissues from control and diabetic rats, respectively (Fig. 2A and B, respectively). In the presence of prazosin, there was also a 7.7-fold (95% confidence limit = 3.8–15.8) shift of the mean noradrenaline concentration-response curve in prostates from insulin-treated diabetic rats ( $P < 0.001$ ) (Fig. 2C). There was no difference between the maximum responses of tissues from control, diabetic or insulin-treated diabetic

rats when they were compared with their corresponding groups in the absence of prazosin.

### 3.4. Effects of the uptake 1 inhibitor, nisoxetine

Nisoxetine (300 nM) produced a significant leftward shift of the mean noradrenaline concentration-response curve in prostates from control (2.8-fold (95% confidence limit = 1.4–6.1);  $P < 0.01$ ) (Fig. 3A), but not diabetic (Fig. 3B), rats. Nisoxetine produced a significant 16.4-fold leftward shift of the mean noradrenaline concentration-response curve of prostates from insulin-treated diabetic rats (95% confidence limit = 9.7–27.7;  $P < 0.001$ ) (Fig. 3C). Nisoxetine had no effect on the maximum responses obtained for tissues from control, diabetic or insulin-treated diabetic rats.

### 3.5. Effects of the $\beta$ -adrenoceptor antagonist, propranolol

Propranolol (1  $\mu$ M) had no significant effect on the noradrenaline concentration-response curve of prostate glands from diabetic rats ( $P > 0.05$ ). However, in the presence of propranolol, there was a small rightward shift of the nor-

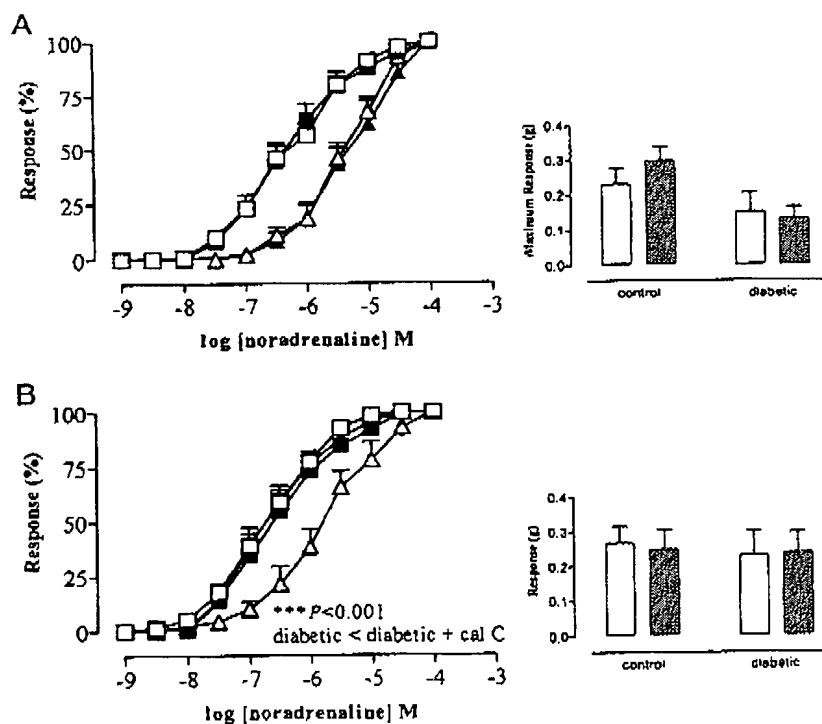


Fig. 6. (A) The effect of bisindolylmaleimide I (500 nM) on the noradrenaline concentration-response curve of prostate glands from control (□,  $n = 6$ ) and diabetic (△,  $n = 6$ ) rats. Solid symbols represent response in the presence of bisindolylmaleimide I. Histogram: maximum response of tissues in the absence (open bars) and presence (cross hatched bars) of bisindolylmaleimide I. Data are expressed as means  $\pm$  S.E.M. (B) The effect of calphostin C (500 nM) on the noradrenaline concentration-response curve of prostate glands from control (□,  $n = 6$ ) and diabetic (△,  $n = 6$ ) rats. Solid symbols represent response in the presence of calphostin C. Histogram: maximum response of tissues in the absence (open bars) and presence (cross hatched bars) of calphostin C. Data are expressed as means  $\pm$  S.E.M. \*\*  $P < 0.001$ , when compared with corresponding curve in the absence of calphostin C.

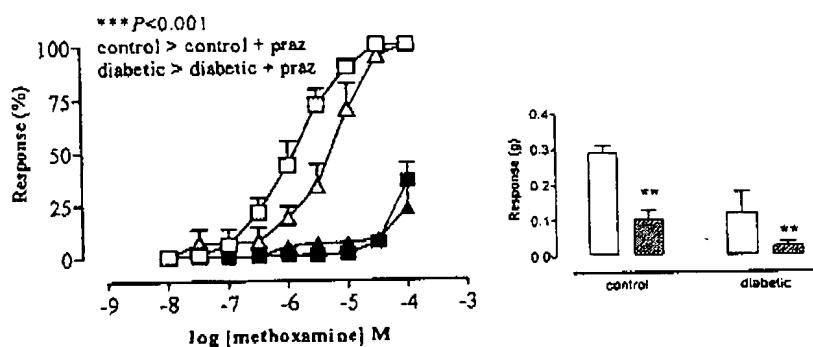


Fig. 7. The effect of prazosin (100 nM) on the methoxamine concentration–response curve of prostate glands from control (□,  $n=5$ ) and diabetic (△,  $n=5$ ) rats. Solid symbols represent response in the presence of prazosin. Histogram: maximum response of tissues in the absence (open bars) and presence (cross-hatched bars) of prazosin. Data are expressed as means  $\pm$  S.E.M.

adrenaline concentration–response curve of prostate glands from control rats ( $P<0.001$ ) (Fig. 4A). Propranolol had no effect on the maximum responses obtained for tissues from control or diabetic rats.

### 3.6. Effects of the muscarinic receptor antagonist, atropine

Lau et al. (2000) have shown that stimulation of muscarinic receptors of the guinea-pig prostate gland are able to enhance noradrenaline mediated contractions. In the present study, atropine (300 nM) had no significant effect on the noradrenaline concentration–response curves of prostate glands from control or diabetic rats ( $P>0.05$ ) (Fig. 4B). Atropine had no effect on the maximum responses obtained for tissues from control or diabetic rats.

### 3.7. Effects of electrical field stimulation

In electrical field stimulated preparations, frequency response curves obtained from control and diabetic rat prostate glands did not differ significantly. Nisoxetine (300 nM) caused a significant 3.6-fold (95% confidence limit = 1.7–16.7) and 4.0-fold (95% confidence limit = 1.5–9.8) leftward shift of the mean frequency–response curve in prostates from control and diabetic rats, respectively ( $P<0.01$ ) (Fig. 5). Nisoxetine did not effect the maximum responses of tissues to stimulation at 20 Hz.

### 3.8. Effects of the protein kinase C inhibitors, bisindolylmaleimide I and calphostin C

Bisindolylmaleimide I (500 nM) did not affect the mean noradrenaline concentration–response curves in prostates from control or diabetic rats (Fig. 6A). Mean maximum responses of control and diabetic tissues were not affected by the addition of bisindolylmaleimide I.

Calphostin C (500 nM) produced a significant 8.6-fold (95% confidence limit = 4.7–15.9) leftward shift of the mean noradrenaline concentration–response curve in tissues

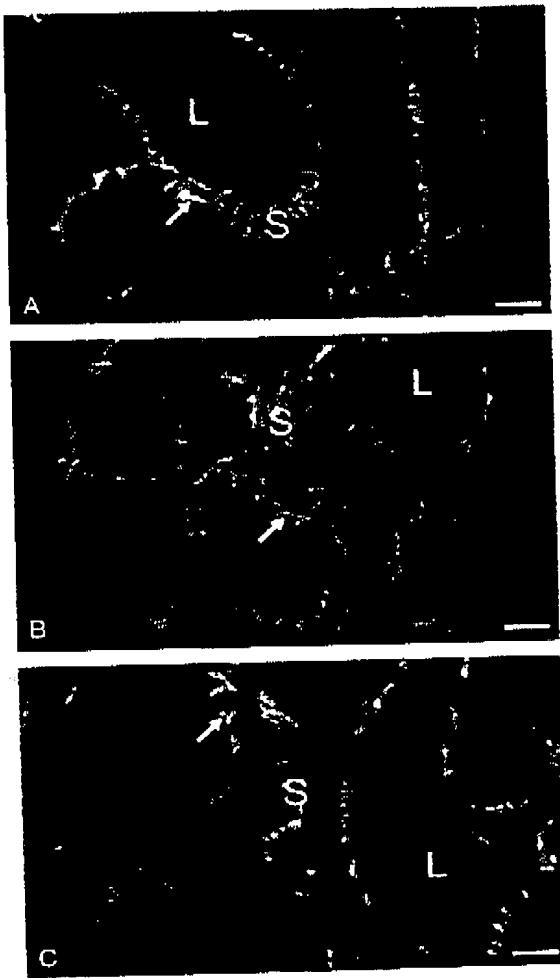


Fig. 8. Photomicrographs showing cross-sections stained for catecholamine fluorescence in prostate glands from (A) control, (B) diabetic and (C) insulin-treated diabetic rats. Arrows indicate fluorescent catecholamine staining neurons.

from diabetic rats ( $P < 0.001$ ) (Fig. 6B). Mean maximum responses of control and diabetic tissues to noradrenaline were not affected by the addition of calphostin C.

### 3.9. Effects of the $\alpha_1$ -agonist, methoxamine

Discrete additions of methoxamine (10 nM–100  $\mu$ M) produced dose-dependent contractions in prostates from control and diabetic rats. The mean methoxamine concentration–response curve obtained in prostates from diabetic rats was shifted 5.0-fold (95% confidence limit = 3.1–8.1) rightward compared with the mean methoxamine concentration–response curve obtained in tissues from control rats ( $P < 0.001$ ) (Fig. 7). Prazosin (100 nM) produced a rightward shift of at least 100-fold in the mean methoxamine concentration–response curves in preparations from both control and diabetic rats ( $P < 0.001$ ) (Fig. 7). Responses to methoxamine in both control and diabetic tissues treated with prazosin did not reach maximum.

### 3.10. Histochemical studies

Catecholamine fluorescence stained dense populations of nerves in the prostatic smooth muscle stroma in between the secretory acini, and was similar in cross sections of prostates taken from control, diabetic and insulin-treated diabetic rats (Fig. 8A–C).

Staining with haematoxylin and eosin displayed similar muscle profiles of prostates taken from 6-week control, diabetic and insulin-treated diabetic rats (results not shown).

## 4. Discussion

In the present study, streptozotocin-diabetic rats exhibited symptoms similar to those observed in humans with uncontrolled type 1, insulin-dependent diabetes mellitus, e.g. hyperglycaemia and weight loss. These observations are consistent with the findings of other studies (Crowe et al., 1987; Gousse et al., 1991; Latifpour et al., 1991; Fukumoto et al., 1993; Nishi et al., 1998).

The mean noradrenaline concentration–response curves of prostate glands from diabetic and insulin-treated diabetic rats were significantly shifted rightward when compared to the curve obtained in tissues from control rats. This effect clearly demonstrates that prostate glands from diabetic rats were less sensitive to noradrenaline when compared to the control, and this sub-sensitivity to noradrenaline was not reversible by chronic insulin treatment. Furthermore, the mean maximum response of prostates from diabetic rats to noradrenaline was significantly reduced when compared with the maximum responses of prostates from control and insulin-treated diabetic rats to noradrenaline. Therefore, diabetes also appears to affect the reactivity of the prostate gland to noradrenaline. In contrast to the diabetes-induced

sub-sensitivity, this decrease in reactivity was reversible with insulin. However, it must also be noted that the reversal of reactivity of prostates from diabetic rats to control levels was not associated with a parallel reversal in prostate weights, despite the chronic administration of insulin reversing some of the effects of streptozotocin treatment, i.e. hyperglycaemia and reduced body weight. Crowe et al. (1987) have suggested that the reduction in prostate size may be due to a decrease in the thickness of the smooth muscle of this tissue. However, in our study, the morphology of prostates taken from 6-week control, diabetic and insulin-treated diabetic rats did not display any obvious differences as seen with histological haematoxylin and eosin staining (results not shown). The  $\alpha_1$ -adrenoceptors, responsible for the contraction of the prostate (Garcia-Paramio et al., 1995; Nishi et al., 1998), and the increase in sympathetic tone seen in benign prostatic hyperplasia (Cooper et al., 1999), are predominantly found in the muscle stroma. Therefore, any alterations in prostatic morphology may not only be related to changes in the size of this gland, but may also affect its ability to contract in response to the addition of exogenous noradrenaline.

In contrast to Crowe et al. (1987), no obvious differences in catecholamine fluorescence were seen between prostates from control, diabetic or insulin-treated diabetic rats. This variance may be due to the fact that the present study used a 6-week streptozotocin-diabetic model whilst that of Crowe et al. (1987) employed an 8-week streptozotocin-diabetic model. This result is consistent with the other findings of the present study. If the diabetic prostate had displayed a decreased catecholamine fluorescence, then we would expect to see an increased sensitivity to noradrenaline due to the loss of uptake 1, a decreased sensitivity of the diabetic prostate gland to electrical field stimulation, and an equal sensitivity to methoxamine of the diabetic prostate. An increase in the innervation of prostatic smooth muscle could explain the sub-sensitivity of the prostate gland seen in this study, but such an increase in innervation was not observed.

Methoxamine is an  $\alpha$ -adrenoceptor agonist with a greater selectivity for the  $\alpha_1$ -subtype and decreased susceptibility to neuronal uptake, compared to noradrenaline (Wikberg, 1978). Prostate glands from diabetic rats were less sensitive to methoxamine than prostates from control rats. This result further supports the notion that subsensitivity is not due to changes in neuronal uptake.

Haynes and Hill (1997) have shown that  $\beta$ -adrenoceptors are involved in the contraction of prostatic smooth muscle in the guinea pig. In addition, previous radioligand receptor binding studies have shown that streptozotocin-diabetic rat prostates exhibit a 45–50% reduction in  $\beta$ -adrenoceptor density (Fukumoto et al., 1993). Similarly, prostate glands from streptozotocin-diabetic rats exhibit decreased densities of muscarinic-receptors of up to 30% (Latifpour et al., 1991; Fukumoto et al., 1993). Previous studies have also shown that cholinergic drugs are able to modify noradrenergic contraction in the isolated guinea-pig prostate gland (Lau et al.,

2000). Such down-regulation in receptors could lead to changes in sympathetic tone. However, in the present study, propranolol and atropine did not shift the noradrenaline concentration-response curve of the diabetic prostate gland towards that of the control, suggesting that changes in  $\beta$ -adrenoceptors or muscarinic receptors play no role in the diabetes-induced changes in the sensitivity of the rat prostate glands to noradrenaline.

Many of the physiological activities and mechanisms of cell proliferation and differentiation in the prostate gland involve protein kinase C activity, which is known to exist in both  $\text{Ca}^{2+}$ -dependent and  $\text{Ca}^{2+}$ -independent forms. In addition to playing a role in the noradrenaline-induced contraction of smooth muscle (Kamimura et al., 2000), protein kinase C is involved in the signal transduction of neurotransmitters, hormones and growth factors that control cell function and proliferation (Garcia-Paramio et al., 1993). In the present study, the protein kinase C inhibitor, bisindolylmaleimide I (500 nM), had no effect on the noradrenaline concentration-response curves of prostates from either control or diabetic rats. In contrast, another protein kinase C inhibitor, calphostin C (500 nM), produced a leftward shift of the noradrenaline concentration-response curve of prostates from diabetic rats, resulting in its return to a position similar to that seen in prostates from control rats. These findings strongly suggest that the sub-sensitivity of the diabetic prostate gland to noradrenaline is due to an alteration in the activity of a protein kinase C isoform other than those blocked by bisindolylmaleimide I. One reason why calphostin C, and not bisindolylmaleimide I, has an effect on the noradrenaline concentration-response curve of prostates from diabetic rats may be explained by their differing modes of action. Bisindolylmaleimide I has a competitive inhibitory action at the ATP binding site of protein kinase C and shows high selectivity for protein kinase C $\alpha$ ,  $\beta_1$ ,  $\beta_2$ ,  $\gamma$ ,  $\delta$  and  $\epsilon$  isoforms (Toullec et al., 1991; Gekeler et al., 1996). Alternatively, calphostin C exerts its inhibitory effect by competing at the binding site of diacylglycerol and phorbol esters, thereby inhibiting the activation of protein kinase C (Kobayashi et al., 1989; Bruns et al., 1991; Gopalakrishna et al., 1992). The concentrations of bisindolylmaleimide I and calphostin C used in the present study have been shown to be selective despite their differing modes of action (Bruns et al., 1991; Gopalakrishna et al., 1992; Kobayashi et al., 1989; Tamaoki et al., 1990; Toullec et al., 1991).

The findings of the present study suggest that the induction of diabetes by streptozotocin results in a reduction in the sensitivity and reactivity of the rat isolated prostate gland to noradrenaline. Interestingly, despite the diabetes-induced decreases in rat body and prostate weight, these reductions do not appear to be due to alterations in the morphology of the gland. Our results suggest that these changes may involve diabetes-induced changes in protein kinase C activity. Similar effects have been seen in other sympathetically innervated tissues following the induction of diabetes (James and Hodgson, 1997).

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The New England Journal of Medicine

# SI UNIT CONVERSION GUIDE

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CONSTITUENT	FLUID	CONVERSION TRAD $\leftrightarrow$ SI → MULT DIV ←	NOMOGRAM PAGE #
Acid Phosphatase	serum	1	
ACTH (adrenocorticotropin)	serum	0.2202	13
Alanine Aminotransferase (ALT) same as SGPT	serum	1	
Albumin	serum	10	14
Alkalase	serum	1	
Alkaline Phosphatase	serum	1	
Amonnia (as NH3)	plasma	0.75872	15
Amylase	serum	1	
Aspartate Aminotransferase (AST) same as SGOT	serum	1	
Bilirubin (Total or Conjugated)	serum	17.1	16
Calcium	serum	0.25	17
Calcium, ionized	serum	0.25	17
Carbon Dioxide	wh bld, plasma, ser	1	32
Chloride	plasma, serum	1	
Cholesterol	plasma, serum	0.02586	18
Complement C3	serum	0.01	19
Complement C4	serum	0.01	20
Cortisol	plasma, serum	27.59	21
Cortisol, Free	urine	2.759	22
Creatinine	plasma, serum	88.4	23
Creatinine	urine	8.84	24
Creatinine Clearance	serum & urine	0.01667	25
Creatine Kinase	serum	1	
Diazepam	plasma, serum	3512	26
Digoxin	serum	1.281	27
Dilantin (Phenytoin)	plasma, serum	3.964	28
Estradiol	plasma, serum	3.671	29
Ethanol	plasma, serum	0.2171	30
Ferritin	plasma, serum	1	
Fibrinogen Split Products (FSP, FDP)	serum	1	
Folate	serum	2.266	31
Follicle Stimulating Hormone (FSH)	plasma, ser, urine	1	
Gases (pO2 & CO2)	arterial, wh bld	0.1333	32
Glucose	plasma, serum	0.05551	33

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CONSTITUENT	FLUID	APPROXIMATE NORMAL RANGE	UNITS	TRADITIONAL UNITS
Gamma Glutamyl Transferase (GGT)	serum	0 - 30 (30°C) 0 - 30 (30°C)	μmol/L μmol/L	
Gases (arterial)				
pCO <sub>2</sub>	N art whole blood art whole blood	35 - 45 7.35 - 7.45	mm Hg pH units	
pH	N art whole blood	7.35 - 7.45	mm Hg	
pO <sub>2</sub>	N art whole blood	75 - 100	mm Hg	
Gastin	plasma, serum	0 - 180	μg/ml	
GGT (Gamma Glutamyl Transferase)	serum	0 - 30 (30°C) 0 - 30 (30°C)	Units/L Units/L	
Globulins, as immunoglobulins				
IgG	N serum	500 - 1200	mg/dL	
IgA	N serum	50 - 350	mg/dL	
IgM	N serum	30 - 250	mg/dL	
IgD	N serum	<6	mg/dL	
IgE	serum	0.5 - 10	UI/mL	
0 - 3 years		50 - 100	UI/mL	
> 3 years	serum	50 - 100	UI/mL	
Glutagon	plasma, serum	70 - 110	μg/dL	
Glucose (fasting)	N plasma, serum	90 - 100	mg/dL	
Glucose	plasma, serum	90 - 100	mg/dL	
Glutamic acid, amino acid fractionation	plasma, serum	0.2 - 2.8	mg/dL	
Glutamine, amino acid fractionation	plasma, serum	6.1 - 10.2	mg/dL	
Glutathione				
Therapeutic	plasma, serum	<10	μg/L	
Toxic	plasma, serum	>20	μg/L	
Glycerol (triglycerides)	serum	<1.5	mg/dL	
Glycine, amino acid fractionation	plasma, serum	0.9 - 4.2	mg/dL	
Cold, therapeutic	serum	300 - 800	μg/dL	
Gold	urine	<500	μg/24 hr	
Growth hormone				
Males (fasting)	plasma, serum	0 - 5	ng/ml	
Females (fasting)	plasma, serum	0 - 10	ng/ml	
Haptoglobin	serum	50 - 220	mg/dL	
HCG (human chorionic gonadotropin)	serum	<10 if not pregnant	mIU/ml	
HCG (human chorionic gonadotropin)	urine	negative	negative	
HDL (high density lipoproteins)	N plasma, serum	30 - 70	mg/dL	
Male	plasma, serum	30 - 50	mg/dL	
Female	whole blood	33 - 43	%	%
Hemostat	N whole blood	39 - 49	%	%
Male				



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# SI UNIT CONVERSION GUIDE

24

twenty-fourth  
edition

# Harper's Biochemistry

Robert K. Murray  
Daryl K. Granner  
Peter A. Mayes  
Victor W. Rodwell



and experts in the field should be consulted whenever results are unusual or in doubt.

#### Effect of Meals and Posture on Concentration of Substances in Blood

**A. Meals:** The usual normal values for blood tests have been determined by assay of "fasting" specimens collected after 8–12 hours of abstinence from food. With few exceptions, water is usually permitted as desired.

Few routine tests are altered from usual fasting values if blood is drawn 3–4 hours after breakfast. When blood is drawn 3–4 hours after lunch, values are more likely to vary from those of the true fasting state. Valid measurement of triacylglycerol (triglyceride) in serum or plasma requires abstinence from food for 10–14 hours.

**B. Posture:** Plasma volume measured in a person who has been supine for several hours is 12–15% greater than in a person who has been up and about or standing for an hour or so. It follows that measurements performed on blood obtained after the subject has been lying down for an hour or more will yield lower values than when blood has been obtained after the same subject has been upright. An intermediate change apparently occurs with sitting.

A tourniquet applied for 1 minute instead of 3 minutes produced the following changes in reported values: total protein, +5%; iron, +6.7%; cholesterol, +5%; AST (SGOT), +9.3%; and bilirubin, +8.4%. Decreases were observed for potassium, -6%; and creatinine, -2.3%.

#### Validity of Laboratory Tests\*

The clinical value of a test is related to its specificity and sensitivity and the incidence of the disease in the population tested.

Sensitivity means percentage of positive results in patients with the disease. The test for phenylketonuria is highly sensitive: a positive result is obtained in all who have the disease (100% sensitivity). The carcinoembryonic antigen (CEA) test has low sensitivity: only 72% of those with carcinoma of the colon test positive when the disease is extensive, and only 20% are positive with early disease. Lower sensitivity occurs in the early stages of many diseases—in contrast to the higher sensitivity in well-established disease.

Specificity means percentage of negative results

among people who do not have the disease. The test for phenylketonuria is highly specific: 99.9% of normal individuals give a negative result. In contrast, the CEA test for carcinoma of the colon has a variable specificity: about 3% of nonsmoking individuals give a false-positive result (97% specificity), whereas 20% of smokers give a false-positive result (80% specificity). The overlap of serum thyroxine levels between hyperthyroid patients and those taking oral contraceptives or those who are pregnant is an example of a change in specificity from that prevailing in a different set of individuals.

The predictive value of a positive test defines the percentage of positive results that are true positives. This is related fundamentally to the incidence of the disease. In a group of patients on a urology service, the incidence of renal disease is higher than in the general population, and the serum creatinine level will have a higher predictive value in that group than for the general population.

Formulas for definitions:

$$\text{Sensitivity} = \frac{\text{True positive}}{\text{True positive} + \text{false negative}} \times 100$$

$$\text{Specificity} = \frac{\text{True negative}}{\text{True negative} + \text{false positive}} \times 100$$

$$\text{Predictive value} = \frac{\text{True positive}}{\text{True positive} + \text{false positive}} \times 100$$

Before ordering a test, attempt to determine whether test sensitivity, specificity, and predictive value are adequate to provide useful information. To be useful, the result should influence diagnosis, prognosis, or therapy; lead to a better understanding of the disease process; and benefit the patient.

#### NORMAL LABORATORY VALUES (Blood [B], Plasma [P], Serum [S], Urine [U])

#### HEMATOLOGY

**Bleeding time:** Ivy method, 1–7 minutes (60–420 seconds). Template method, 3–9 minutes (180–540 seconds).

**Cellular measurements of red cells:** Average diameter = 7.3  $\mu\text{m}$  (5.5–8.8  $\mu\text{m}$ ). Mean corpuscular volume (MCV): Men, 80–94 fL; women, 81–99 fL (by Coulter counter). Mean corpuscular hemoglobin (MCH): 27–32 pg. Mean corpuscular hemoglobin concentration (MCHC): 32–36 g/dL red blood cells (32–36%).

**Clot retraction**  
6–24 hours. N

**Fibrinogen split**

**Fragility of red**  
complete at 0.

**Hematocrit (P**  
women, 37–41

**Hemoglobin:** [  
mmol/L as H  
(1.86–2.48 mm)

**Partial thrombo-**  
onds.

**Platelets:** 150,000

**Prothrombin:** [  
Normalized R]

**Red blood count**  
(4.5–6.2  $\times$  10<sup>12</sup>/  
(4.5–5.5  $\times$  10<sup>12</sup>/L)

**Reticulocytes:** 0

**Sedimentation rate**  
(gren); 0–10 mm

**White blood count**  
5000–10,000/ $\mu\text{l}$

**Myelocytes**

**Juvenile neutrophils**

**Band neutrophils**

**Segmented neutrophils**

**Lymphocytes**

**Eosinophils**

**Basophils**

**Monocytes**

**Lymphocytes:**

**B cell**

**T cell**

**Suppressor**

**Helper**

**H:S**

#### CLINICAL CHEMISTRY

**Acetone and acetoacetate** mg/L).

**Adrenal hormones**: Aldosterone: [I]  
Values vary

**Catecholamines**

**nephrine, < 1**

**nephrine, < 1**

**Cortisol, free:**  $\mu\text{mol}/\text{d}$ .

**11,17-Hydroxyprogesterone** in women, 4–8

\*This section is an abridged version of an article by Krieg AF, Gambino R, Galen RS: Why are clinical laboratory tests performed? When are they valid? JAMA 1975;233:76. Reprinted from the *Journal of the American Medical Association*. Copyright © 1975 by American Medical Association. See also Galen RS, Gambino SR: *Beyond Normality: The Predictive Value and Efficiency of Medical Diagnosis*. Wiley, 1975.

disease. The test is 99.9% of normal. In contrast, the test has a variable result (80% of individuals give false positive result (80% thyroxine levels those taking oral thyroid are an example prevailing in

test defines the true positives. Incidence of the urology service, higher than in the creatinine level that group than

$\text{_____} \times 100$   
positive  
 $\text{_____} \times 100$   
negative  
 $\text{_____} \times 100$   
positive

to determine and predictive information. To diagnosis, prognosis, understanding of patient.

LUES  
in [S],

minutes (60–420)  
minutes (180–

Average diameter of corpuscular men, 81–99 fL.  
Corpuscular hemoglobin: 36 g/dL red

**Clot retraction:** Begins in 1–3 hours; complete in 6–24 hours. No clot lysis in 24 hours.

**Fibrinogen split products:** Negative > 1:4 dilution.

**Fragility of red cells:** Begins at 0.45–0.38% NaCl; complete at 0.36–0.3% NaCl.

**Hematocrit (PCV):** Men, 40–52% (0.4–0.52); women, 37–47% (0.37–0.47).

**Hemoglobin:** [B] Men, 14–18 g/dL (2.09–2.79 mmol/L as Hb tetramer); women, 12–16 g/dL (1.86–2.48 mmol/L). [S] 2–3 mg/dL.

**Partial thromboplastin time:** Activated, 25–37 seconds.

**Platelets:** 150,000–400,000/ $\mu\text{L}$  ( $0.15\text{--}0.4 \times 10^{12}/\text{L}$ ).

**Prothrombin:** [P] 11–14.5 seconds. International Normalized Ratio (INR): [P] 2.0–3.0.

**Red blood count (RBC):** Men, 4.5–6.2 million/ $\mu\text{L}$  ( $4.5\text{--}6.2 \times 10^{12}/\text{L}$ ); women, 4–5.5 million/ $\mu\text{L}$  ( $4\text{--}5.5 \times 10^{12}/\text{L}$ ).

**Reticulocytes:** 0.2–2% of red cells.

**Sedimentation rate:** Less than 20 mm/h (Westergren); 0–10 mm/h (Wintrobe).

**White blood count (WBC) and differential:** 5000–10,000/ $\mu\text{L}$  ( $5\text{--}10 \times 10^9/\text{L}$ ).

Myelocytes	0 %
Juvenile neutrophils	0 %
Band neutrophils	0–5 %
Segmented neutrophils	40–60%
Lymphocytes	20–40%
Eosinophils	1–3 %
Basophils	0–1 %
Monocytes	4–8 %
Lymphocytes: Total, 1500–4000/ $\mu\text{L}$	
B cell	5–25%
T cell	60–88%
Suppressor	10–43%
Helper	32–66%
H.S.	> 1

## CLINICAL CHEMISTRY

**Acetone and acetoacetate:** [S] 0.3–2 mg/dL (3–20 mg/L).

**Adrenal hormones and metabolites:**

- Aldosterone: [U] 2–26  $\mu\text{g}/24\text{ h}$  (5.5–72 nmol/d). Values vary with sodium and potassium intake.
- Catecholamines: [U] Total, < 100  $\mu\text{g}/24\text{ h}$ . Epinephrine, < 10  $\mu\text{g}/24\text{ h}$  (< 100 nmol/d); norepinephrine, < 100  $\mu\text{g}/24\text{ h}$  (< 590 nmol/d).
- Cortisol, free: [U] 20–100  $\mu\text{g}/24\text{ h}$  (0.55–2.76  $\mu\text{mol}/\text{d}$ ).
- 11,17-Hydroxycorticoids: [U] Men, 4–12 mg/24 h; women, 4–8 mg/24 h.

17-Ketosteroids: [U] Under 8 years, 0–2 mg/24 h; adolescents, 2–20 mg/24 h. (1 mg = 3.5  $\mu\text{mol}/\text{d}$ )

Metanephrine: [U] < 1.3 mg/24 h (< 6.6  $\mu\text{mol}/\text{d}$ ) or < 2.2  $\mu\text{g}/\text{mg}$  creatinine.

Vanillylmandelic acid (VMA): [U] Up to 7 mg/24 h (< 35  $\mu\text{mol}/\text{d}$ ).

## Aminotransferases:

Aspartate aminotransferase (AST; SGOT): 0–41 IU/L at 37 °C.

Alanine aminotransferase (ALT; SGPT): 0–45 IU/L at 37 °C.

**Ammonia:** [P] (as NH<sub>3</sub>) 10–80  $\mu\text{g}/\text{dL}$  (5–50  $\mu\text{mol}/\text{L}$ ).

**Amylase:** [S] 80–180 units/dL (Somogyi).

**$\alpha_1$ -Antitrypsin:** [S] > 180 mg/dL.

**Ascorbic acid:** [P] 0.4–1.5 mg/dL (23–85  $\mu\text{mol}/\text{L}$ ).

**Bicarbonate:** [S] 24–28 meq/L (24–28 mmol/L).

**Bilirubin:** [S] Total, 0.2–1.2 mg/dL (3.5–20.5  $\mu\text{mol}/\text{L}$ ). Direct (conjugated), 0.1–0.4 mg/dL (< 7  $\mu\text{mol}/\text{L}$ ). Indirect, 0.2–0.7 mg/dL (< 12  $\mu\text{mol}/\text{L}$ ).

**Calcium:** [S] 8.5–10.3 mg/dL (2.1–2.6 mmol/L). Values vary with albumin concentration.

**Calcium, ionized:** [S] 4.25–5.25 mg/dL; 2.1–2.6 meq/L (1.05–1.3 mmol/L).

**$\beta$ -Carotene:** [S, fasting] 50–300  $\mu\text{g}/\text{dL}$  (0.9–5.58  $\mu\text{mol}/\text{L}$ ).

**Ceruloplasmin:** [S] 25–43 mg/dL (1.7–2.9  $\mu\text{mol}/\text{L}$ ).

**Chloride:** [S or P] 96–106 meq/L (96–106 mmol/L).

**Cholesterol:** [S or P] 150–220 mg/dL (3.9–5.72 mmol/L). (See Lipid fractions, below.)

**Cholesteryl esters:** [S] 65–75% of total cholesterol.

**CO<sub>2</sub> content:** [S or P] 24–29 meq/L (24–29 mmol/L).

**Complement:** [S] C3 ( $\beta_{1C}$ ), 90–250 mg/dL. C4 ( $\beta_{1E}$ ), 10–60 mg/dL. Total (CH<sub>50</sub>), 75–160 mg/dL.

**Copper:** [S or P] 100–200  $\mu\text{g}/\text{dL}$  (16–31  $\mu\text{mol}/\text{L}$ ).

**Cortisol:** [P] 8:00 AM, 5–25  $\mu\text{g}/\text{dL}$  (138–690 nmol/L); 8:00 PM < 10  $\mu\text{g}/\text{dL}$  (275 nmol/L).

**Creatine kinase (CK):** [S] 10–50 IU/L at 30 °C.

**Creatine kinase isoenzymes:** BB, 0%; MB, 0–3%; MM, 97–100%.

**Creatinine:** [S or P] 0.7–1.5 mg/dL (62–132  $\mu\text{mol}/\text{L}$ ).

**Cyanocobalamin:** [S] 200 pg/mL (148 pmol/L).

**Epinephrine:** [P] Supine, < 100 pg/mL (< 550 pmol/L).

**Fecal fat:** < 30% dry weight.

**Ferritin:** [S] Adult women, 20–120 ng/mL; men, 30–300 ng/mL. Child to 15 years, 7–140 ng/mL.

**Folic acid:** [S] 2–20 ng/mL (4.5–45 nmol/L). [RBC] > 140 ng/mL (> 318 nmol/L).

**Glucose:** [S or P] 65–110 mg/dL (3.6–6.1 mmol/L).

**γ-Glutamyl transpeptidase:** [S] < 30 units/L at 30 °C.

**Haptoglobin:** [S] 40–170 mg of hemoglobin-binding capacity.

**Iron:** [S] 50–175 µg/dL (9–31.3 µmol/L).

**Iron-binding capacity:** [S] Total, 250–410 µg/dL (44.7–73.4 µmol/L). Percent saturation, 20–55%.

**Lactate:** [B, special handling] Venous, 4–16 mg/dL (0.44–1.8 mmol/L).

**Lactate dehydrogenase (LDH):** [S] 55–140 IU/L at 30 °C; SMA, 100–225 IU/L at 37 °C; SMAC, 60–200 IU/L at 37 °C.

**Lead:** [U] < 80 µg/24 h (< 0.4 µmol/d).

**Lipase:** [S] < 150 units/L.

**Lipid fractions:** [S or P] Desirable levels: HDL cholesterol, > 40 mg/dL; LDL cholesterol, < 180 mg/dL; VLDL cholesterol, < 40 mg/dL. (To convert to mmol/L, multiply by 0.026.)

**Lipids, total:** [S] 450–1000 mg/dL (4.5–10 g/L).

**Magnesium:** [S or P] 1.8–3 mg/dL (0.75–1.25 mmol/L).

**Norepinephrine:** [P] Supine, < 500 pg/mL (< 3 nmol/L).

**Osmolality:** [S] 280–296 mosm/kg water (280–296 mmol/kg water).

#### Oxygen:

Capacity: [B] 16–24 vol%. Values vary with hemoglobin concentration.

Arterial content: [B] 15–23 vol%. Values vary with hemoglobin concentration.

Arterial % saturation: 94–100% of capacity.

Arterial PO<sub>2</sub> (PaO<sub>2</sub>): 80–100 mm Hg (10.67–13.33 kPa) (sea level). Values vary with age.

**PaCO<sub>2</sub>:** [B, arterial] 35–45 mm Hg (4.7–6 kPa).

**pH:** [B, arterial] 7.35–7.45 (H<sup>+</sup> 44.7–45.5 nmol/L).

**Phosphatase, acid:** [S] 1–5 units (King-Armstrong), 0.1–0.63 units (Bessey-Lowry).

**Phosphatase, alkaline:** [S] Adults, 5–13 units (King-Armstrong); 0.8–2.3 (Bessey-Lowry); SMA, 30–85 IU/L at 37 °C; SMAC, 30–115 IU/L at 37 °C.

**Phospholipid:** [S] 145–200 mg/dL (1.45–2 g/dL).

**Phosphorus, inorganic:** [S, fasting] 3–4.5 mg/dL (1–1.5 mmol/L).

#### Porphyrins:

Delta-aminolevulinic acid: [U] 1.5–7.5 mg/24 h (11–57 µmol/d).

**Coproporphyrin:** [U] < 230 µg/24 h (< 350 nmol/d).

**Uroporphyrin:** [U] < 50 µg/24 h (< 60 nmol/d).

**Porphobilinogen:** [U] < 2 mg/24 h (< 8.8 µmol/d).

**Potassium:** [S or P] 3.5–5 meq/L (3.5–5 mmol/L).

#### Protein:

Total: [S] 6–8 g/dL (60–80 g/L).

Albumin: [S] 3.5–5.5 g/dL (35–55 g/L).

Globulin: [S] 2–3.6 g/dL (20–36 g/L).

Fibrinogen: [P] 0.2–0.6 g/dL (2–6 g/L).

**Pyruvate:** [B] 0.6–1 mg/dL (70–114 µmol/L).

**Serotonin:** [B] 5–20 µg/dL (0.2–1.14 µmol/L).

**Sodium:** [S or P] 136–145 meq/L (136–145 mmol/L).

**Specific gravity:** [B] 1.056 (varies with hemoglobin and protein concentration). [S] 1.0254–1.0288 (varies with protein concentration).

**Sulfate:** [S or P] As sulfur, 0.5–1.5 mg/dL (156–468 µmol/L).

**Transferrin:** [S] 200–400 mg/dL (23–45 µmol/L).

**Triglycerides:** [S] < 165 mg/dL (1.9 mmol/L). (See Lipid fractions, above.)

**Urea nitrogen:** [S or P] 8–25 mg/dL (2.9–8.9 mmol/L). Do not use anticoagulant containing ammonium oxalate.

**Uric acid:** [S or P] Men, 3–9 mg/dL (0.18–0.54 mmol/L); women, 2.5–7.5 mg/dL (0.15–0.45 mmol/L).

**Urobilinogen:** [U] 0–2.5 mg/24 h (70–470 µmol/d).

**Urobilinogen, fecal:** 40–280 mg/24 h (70–470 µmol/d).

**Vitamin A:** [S] 15–60 µg/dL (0.53–2.1 µmol/L).

**Vitamin B<sub>12</sub>:** [S] > 200 pg/mL (> 148 pmol/L).

**Vitamin D:** [S] Cholecalciferol (D<sub>3</sub>): 25-Hydroxycholecalciferol, 8–55 ng/mL (19.4–137 nmol/L); 1,25-dihydroxycholecalciferol, 26–65 pg/mL (62–155 pmol/L); 24,25-dihydroxycholecalciferol, 1–5 ng/mL (2.4–12 nmol/L).

**Volume, blood (Evans blue dye method):** Adults, 2990–6980 mL. Women, 46.3–85.5 mL/kg; men, 66.2–97.7 mL/kg.

**Zinc:** [S] 50–150 µg/dL (7.65–22.95 µmol/L).

## HORMONES, SERUM OR PLASMA

#### Adrenal:

Aldosterone: [P] Supine, normal salt intake, 2–9 ng/dL (56–250 pmol/L); increased when upright.

Cortisol: [S] 8:00 AM, 5–20 µg/dL (0.14–0.55 µmol/L); 8:00 PM, < 10 µg/dL (0.28 µmol/L).

Deoxycortisol: [S] (> 0.2 µmol/L).

Dopamine: [P] <

Epinephrine: [P] <

Norepinephrine: [

#### Gonad:

Testosterone, free 0.3–2 ng/dL. (1

Testosterone, total adult men, 30–20–80 ng/dL; h

Estradiol (E<sub>2</sub>): [S]

pg/mL; wome

24–68 pg/mL;

21–30 days, 72 = 3.6 pmol/L.)

Progesterone: [S] mg/mL; luteal > 24 ng/mL; m

#### Islets:

Insulin: [S] 4–25

C-peptide: [S] 0.9

Glucagon: [S, fas

#### Kidney:

Renin activity: sodium intake: 3–6 ng/mL/h. ng/mL/h; stand

#### Parathyroid:

Parathyroid method and anti cium.

#### Pituitary:

Growth hormone (46–465 pmol/L)

Thyroid-stimulat µU/mL.

Follicle-stimulati

tal, 2–12 mIU adult women, menopausal, 3

Luteinizing hor mIU/mL; adu women, < 3 menopausal, >

Corticotropin (A 100 pg/mL (22

Prolactin: [S] 1– Somatomedin C:

Antidiuretic hor Serum osmol: > 290 mosm/k

#### Placenta:

Estriol (E<sub>3</sub>): [S] < 0.2 µg/dL (<

Chorionic gonad < 9 mIU/mL; tion, > 10 mIU

*DORLAND'S ILLUSTRATED*

*Medical  
Dictionary*

*Twenty-fifth Edition*

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1 from the stratum  
2. a collection.  
3. a nail-like part  
grown nail; see un-  
*lis cer'ebri*, cal-

aw," "talon"] the

of] a hooved mam-

*ingula* hoof + *grad-*  
ie tips of one or two  
of certain quadrup-

g one.

*uni-* + *L. articulus*

*axis axis*] 1. hav-  
n an axial direction

*basis base*] having

+ *L. camera* cham-  
partment.

+ *L. cellula* cell]

*e bacteria*.

[*L. centrum* center]

center.

*entral*.

*at head*] having one

r with a single com-  
*chain theory*, under

*ingle*] the obsolete

l of venereal virus.

*(cornis)* having only

with only one cusp.

having only one cusp,

-al) flowing in only

wing one flagellum

or pertaining to a

*L. foratus* pierced)

- + *L. geminus* twin)

in of a pair.

pertaining to a single

pertaining to or at

*imigravida*.

wing only one layer or

+ *L. latus* side)] al-

only one lobe; consist-

+ *L. loculus*] having

; only one mode.

*trek'to-mizd*) having

pertaining to a single

ed) having but one

leate.

+ *L. oculus* eye)] per-

eye.

cess of healing; the re-

n bone or between the

*faulty u.*, an un-

healing by first inten-

tion. *vicious u.*, union of the ends of a fractured bone so as to produce deformity.

**uniovular** (*u'*ne-ov'u-lar) [*uni-* + *L. ovum* egg] arising from one ovum; said of monozygotic twin pregnancies.

**unipara** (*u-nip'ah-rah*) primipara.

**uniparental** (*u'*ne-pah-ren'tal) pertaining to one of the parents only.

**uniparous** (*u-nip'ah-rus*) [*uni-* + *L. parere* to bring forth, produce] 1. producing only one ovum or offspring at one time. 2. primiparous.

**unipolar** (*u'*nl-po'lar) [*uni-* + *L. polus* pole] having but a single pole or process, as a nerve cell.

**unipotency** (*u'*nl-po'ten-se) [*L. unus* one + *potentia* power] the ability of a part to develop in one manner only, or of a cell to develop into only one type of cell.

**unipotent** (*u-nip'o-tent*) unipotential.

**unipotential** (*u'*nl-po-tent'shal) [*uni-* + *L. potens* able] capable in one way only; said of cells which have had their fates determined and can give rise to cells of one order only. Cf. *totipotential*.

**unirritable** (*un-ir'i-tah-b'l*) not irritable; not capable of being stimulated.

**uniseptate** (*u'*ne-sep'tat) having only one septum.

**unisexual** (*u'*nl-seks'u-al) [*uni-* + *L. sexus* sex] of only one sex; having the sexual organs of one sex only.

**unit** (*u'nit*) [*L. unus* one] 1. a single thing. 2. a quantity assumed as a standard of measurement. 3. a gene.

**alexicin u.**, the smallest quantity of alexicin serum required to lyse a given amount of red blood cells in the presence of an excess of hemolytic serum. **Allen-Doisy u.**, see *mouse u.* and *rat u.* **amboceptor u.**, the least quantity of amboceptor with which a definite amount of red blood cells will be lysed by an excess of complement. **American Drug Manufacturers' Association u.**, one tenth of the Steenbock unit. **Angström u.**, the unit of wavelength of electromagnetic and corpuscular radiations, equal to  $10^{-7}$  mm. Called also *angstrom*. Abbreviated A., A., or A.U. **Ansbacher u.**, a unit of vitamin K dosage.

**antigen u.**, the least quantity of antigen which will fix one unit of complement so as to prevent hemolysis. **antitoxic u.**, a unit for expressing the strength of an antitoxin. The unit of diphtheria antitoxin is approximately the amount of antitoxin which will preserve the life of a guinea pig weighing 250 gm. for at least four days after it is injected subcutaneously with a mixture of 100 times the minimum lethal dose of diphtheria toxin. Practically, it is the equivalent of a standard unit preserved in Washington. The unit of tetanus antitoxin is approximately ten times the amount of tetanus antitoxin which will preserve the life of a guinea pig weighing 350 gm. for at least ninety-six hours after injection of a mixture with 100 minimum lethal doses of tetanus toxin. The U.S. Public Health Service unit for scarlet fever antitoxin neutralizes 50 skin test doses of scarlet fever toxin. Abbreviated A.E. (Ger. *antitoxineinheit*).

**atomic mass u.**, the unit mass equal to  $\frac{1}{12}$  the mass of the nuclide of carbon-12; abbreviated amu.

**atomic weight u.**, atomic mass u. **avena u.**, the amount of auxin which applied to one side of the tip of an oat sprout will cause a curvature of 10 degrees.

**Behnken's u.**, a unit of roentgen-ray exposure, being that quantity which, when applied in 1 cc. of air at 18° C. and 760 mm. Hg of pressure, engenders sufficient electric conductivity to equal one electrostatic unit, as measured by the saturation current. **Bodansky u.**, the quantity of phosphatase in 100 ml. of serum required to liberate 1 mg. of phosphorus as phosphate ion from sodium  $\beta$ -glycerophosphate in 1 hour at 37° C. and under other standardized conditions. **British thermal u.**, the amount of heat necessary to raise the temperature of 1 pound of water from 39° F. to 40° F., abbreviated B.T.U. **cat u.**, that amount of digitalis calculated per kilogram of weight of a cat which is just sufficient to kill when slowly and continuously injected into the vein (Hatcher). **C. G. S. u.**, any unit in the centimeter-gram-second system. **Clauberg's u.**, a unit of progestin which is essentially one half of a Corner-Alen unit. **clinical u.**, a unit of estrogenic activity equal to approximately one sixth of the international unit. **Collip u.**, a unit of dosage of parathyroid extract: it is

one one-hundredth of the amount required to increase by 5 mg. the quantity of calcium in 100 ml. of blood at the end of fifteen hours in a dog of 20 kg. weight. **complement u.**, the least quantity of complement which will hemolyze a definite amount of red blood cells in the presence of an amboceptor unit. **Corner-Alen u.**, a unit of progestin dosage. **coronary care u.**, a specially designed and equipped hospital area containing a small number of private rooms, with all facilities necessary for constant observation and possible emergency treatment of patients with severe heart disease. **Craw u.**, the amount of veratrum viride which causes cardiac arrest in the crustacean *Daphnia magna*. **u. of current**, see *ampere*. **dental u.**, 1. a single tooth. 2. a piece of dental equipment providing several services (electrical, air, gas, etc.). **digitalis u.**, any of several units once used in bioassay of digitalis preparations and named according to the animal in which it was determined, as cat unit, etc.

**electromagnetic u's**, that system of units based on the fundamental definition of a unit magnetic pole as one which will repel an exactly similar pole with a force of one dyne when the poles are 1 cm. apart. **electrostatic u's**, that system of units based on the fundamental definition of a unit charge as one which will repel an equal and like charge with a force of one dyne when the two charges are 1 cm. apart in a vacuum. Abbreviated E.S.E. (Ger. *elektrostatische einheit*) and e.s.u.

**enzyme u.**, that amount of an enzyme which will catalyze the transformation of 1 micromole of substrate (or of 1 microequivalent of the substrate group when more than one bond is attacked, as in polysaccharides) per minute under standard conditions of temperature (30° C.), optimal pH, and optimal substrate concentration.

**Felton's u.**, a mouse protective unit of antipneumococcal serum; it is that quantity of antibody capable of protecting a white Swiss mouse against one million fatal doses of a standard pneumococcus culture of the corresponding type. Frequently, it is considered to be the equivalent of the National Institutes of Health control serum (P-11). **Florey u.** (obs.), Oxford u.

**u. of force**, see *dyne*. **Hampson u.**, a unit of roentgen-ray exposure; it is one quarter of the erythema dose. **Hanson u.**, a unit of parathyroid extract, being one one-hundredth of the amount required to increase by 1 mg. the amount of calcium in the blood serum of a parathyroidectomized dog weighing 15 kg.

**u. of heat**, the quantity of heat required to raise the temperature of a kilogram of water 1° C. See *calorie* and *British thermal u.* **hemolytic u.**, the amount of inactivated immune serum which, in the presence of complement, will completely hemolyze 1 ml. of a 5 percent suspension of washed red blood cells.

**hemorrhagin u.**, the amount of snake venom necessary to produce hemorrhages in the vascular network of a three-day-old chick embryo. **Holzknecht u.**, a unit of roentgen-ray exposure equal to one-fifth the erythema dose. Abbreviated H.

**intensive care u.**, a hospital unit in which are concentrated special equipment and skilled personnel for the care of seriously ill patients requiring immediate and continuous attention; abbreviated ICU.

**International u.**, a unit of biological material, as of enzymes, hormones, vitamins, etc., established by the International Conference for the Unification of Formulas. **international u. of estrogenic activity**, the estrogen-producing activity represented in 0.1 microgram of the international standard estrone.

**international u. of gonadotrophic activity**, the specific gonadotrophic activity of 0.1 mg. of the standard material preserved at and distributed from the National Institute for Medical Research, Hampstead, London. It is derived from pregnancy urine and it is approximately the amount required to produce cornification of the vaginal epithelium of the immature rat.

**international insulin u.**, one twenty-second of a milligram of the pure crystalline product now adopted as the standard. **international u. of male hormone**, the androgenic activity represented in 0.1 mg. of crystalline androsterone.

**international u. of penicillin**, the specific penicillin activity contained in 0.6 microgram of the international standard sodium salt of II or G penicillin.

**international u. of vitamin A**, activity equivalent to 0.6 micrograms of pure beta-carotene. **interna-**

# **Martindale**

## The complete drug reference

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# Antidiabetics

This chapter describes diabetes mellitus and its management with antidiabetics. The oral drugs included in this chapter are classified in Table 1, below; insulin, which is given parenterally, is discussed on p.324 and classified in Table 2, p.326.

## Diabetes mellitus

Diabetes mellitus is a group of disorders of carbohydrate metabolism in which the action of insulin is diminished or absent through altered secretion, decreased insulin activity, or a combination of both factors. It is characterised by hyperglycaemia. As the disease progresses tissue or vascular damage ensues leading to severe complications such as retinopathy, nephropathy, neuropathy, cardiovascular disease, and foot ulceration.

Diabetes mellitus may be categorised into several types but the two major types are type 1 (insulin-dependent diabetes mellitus; IDDM) and type 2 (non-insulin-dependent diabetes mellitus; NIDDM). The term juvenile-onset diabetes has sometimes been used for type 1 and maturity-onset diabetes for type 2. Malnutrition-related diabetes is no longer considered a separate entity (see Effects of Cassava, under Starch, p.1380).

Type 1 diabetes mellitus is present in patients who have little or no endogenous insulin secretory capacity and who therefore require exogenous insulin therapy for survival. This form of the disease has an auto-immune basis in most cases, and usually develops before adulthood. The associated hypoinsulinaemia and hyperglucagonaemia put such patients at risk of ketosis and ketoacidosis.

Type 2 diabetes mellitus the disease typically develops in later life. Insulin secretion may appear normal or even excessive (and type 2 patients are thus less prone to ketosis) but it is insufficient to compensate for insulin resistance. Obesity is present in the majority of type 2 patients; non-obese patients tend to have low insulin secretory capacity (although not as low as in type 1 diabetes) rather than appreciable insulin resistance.

**Diagnosis of diabetes mellitus.** Diagnosis is based upon blood-glucose concentrations exceeding set values under specified conditions.<sup>14</sup> Diabetes mellitus is likely if the glucose concentration in a random sample of venous plasma is 11.1 mmol or more per litre. If there are accompanying symptoms of increased thirst and urine volume, recurrent infections and weight loss, the presence of marked hyperglycaemia is considered diagnostic of diabetes. In the absence of symptoms or if the elevation of blood-glucose concentration is less marked (more often the case with type 2 than with type 1 diabetes), the diagnosis needs to be confirmed either by repeated sampling or by an oral glucose tolerance test (OGTT). This test consists of an overnight fast followed by measurement of the fasting blood-glucose concentration, then the administration of a 75-g oral glucose load (in children 0.75 g per kg body-weight up to a maximum of 75 g), and further measurement of the blood-glucose concen-

tration two hours later. Diagnostic values for measurements in venous whole blood are greater than or equal to 6.1 mmol per litre for the fasting state and 10.0 mmol per litre after the glucose load. The corresponding values for capillary whole blood are 6.1 and 11.1 mmol per litre, and for venous plasma 7.0 and 11.1 mmol per litre.<sup>5</sup> There has been some confusion at an international level as to whether the glucose load should be 75 g of the anhydrous form or the monohydrate. Sources at WHO have therefore suggested that the form should be standardised as 75 g of anhydrous glucose (anhydrous dextrose), which would be equivalent to 82.5 g of the monohydrate (glucose BP; dextrose monohydrate). The threshold for the diagnosis of diabetes has recently been lowered from a fasting plasma glucose concentration of 7.8 mmol per litre,<sup>3</sup> to reflect an increased risk of microvascular disease in patients with concentrations of 7.0 mmol per litre or more. However, there has been some concern about the lack of agreement between these newer criteria and the previous ones, which may result in alterations in prevalence of diabetes.<sup>6,7</sup>

Other diagnostic methods, such as measurement of glycosylated haemoglobin have been investigated.<sup>8</sup> There is also some interest in using antibodies to insulin, to islet cells, or to the enzyme glutamic acid decarboxylase, as predictive tests for those patients likely to develop diabetes mellitus.<sup>9</sup>

Once the presence of diabetes has been confirmed the distinction between type 1 and type 2 is made on clinical grounds.

## Management of diabetes mellitus.

- **DIETARY MODIFICATION.** Dietary control is important in both type 1 and type 2 diabetes, and in the latter, possibly in association with increased exercise (see below), may sometimes correct the condition, at least temporarily. Reducing dietary sugar is not the only aim. Correction of obesity is desirable in all patients and in those with type 2 diabetes it will remove one of the factors associated with insulin resistance. Anorectic drugs are not effective in promoting weight loss in these patients.<sup>10</sup> A high fibre intake may also lower blood-glucose concentrations and additional fibre is sometimes taken in the form of guar gum (see p.324). The influence of diet on diabetes is such that all diabetic patients need to be aware of the composition of foods and to be able to make adjustments to their diet, especially to counteract treatment-induced hypoglycaemia. Controversy continues, however, as to the optimum composition of the diet in diabetics, and in particular as to what the relative contribution of calories from fat and from carbohydrate should be.
- **EXERCISE.** All diabetic patients should be encouraged to exercise, according to their age and physical capability.<sup>5</sup> Exercise improves metabolism and enhances the action of insulin on the tissues. It is also a useful component of any weight reduction programme although diet is more effective in promoting weight loss and metabolic control.<sup>10</sup>
- **ORAL ANTIDIABETICS.** If patients with type 2 diabetes have not achieved suitable control after about 3 months of dietary modification and increased physical activity, oral antidiabetics (oral hypoglycaemics) may be tried. The two major classes are the *sulfonylureas* and the *biguanides*. Sulfonylureas act mainly by increasing endogenous insulin secretion, while biguanides act chiefly by decreasing hepatic gluconeogenesis and increasing peripheral utilisation of glucose. Both types function only in the presence of some endogenous insulin production. More recently developed classes of oral antidiabetics include the *alpha-glucosidase inhibitors*, the *meglitinides*, and the *thiazolidinediones* (see Table 1). Alpha-glucosidase inhibitors act by delaying the absorption of glucose from the gastrointestinal tract; meglitinides increase endogenous insulin secretion; and thiazolidinediones appear to increase insulin sensitivity.

Oral treatment of type 2 diabetes in non-obese patients is usually begun with a sulfonylurea. Chlorpropamide and glibenclamide have long half-lives and hence an increased tendency to cause hypoglycaemia, although a large study<sup>11</sup> reported that hypoglycaemic episodes were less frequent with chlorpropamide than glibenclamide. These 2 drugs are best avoided in the elderly;

a sulfonylurea with a short half-life, such as gliclazide, glibidone, or tolbutamide, should be used instead.

There is evidence that the use of low-dose sulfonylurea therapy in patients with diagnosed type 2 diabetes but near-normoglycaemia due to early remission (the so-called honeymoon period) can delay the onset of hyperglycaemia.<sup>12</sup>

Sulfonylureas can cause weight gain and obese patients are preferably treated with the biguanide metformin rather than with a sulfonylurea. Results from the UK Prospective Diabetes Study (UKPDS) have suggested that the use of metformin to provide intensive blood-glucose control in overweight diabetic patients substantially reduced the risk of diabetes-related endpoints such as myocardial infarction, stroke, amputation, renal failure, blindness, and death.<sup>13</sup> Metformin is as effective as the sulfonylureas in terms of blood-glucose control and is less likely to cause hypoglycaemia,<sup>13</sup> but has a rare tendency to cause lactic acidosis in patients with renal impairment, in whom it should not be used.

Drug treatment may also involve an alpha-glucosidase inhibitor such as acarbose or miglitol. These have a small but significant effect in lowering blood glucose and do not cause hypoglycaemia when used alone, but they can cause intolerable gastrointestinal effects. Meglitinides, such as repaglinide and nateglinide are a new chemical class of antidiabetics which act similarly to sulfonylureas; repaglinide has a rapid onset and short duration of action and is administered with meals. The thiazolidinediones, such as pioglitazone or rosiglitazone, appear to increase insulin sensitivity and have been the subject of much interest for type 2 diabetes and insulin resistance, but hepatotoxicity has limited the use of troglitazone.

Should treatment with one of the oral antidiabetics fail then a different type or in some instances combinations of different types may produce improvement. Alarming evidence<sup>13</sup> of an increased risk of death in UKPDS patients given intensive therapy with metformin in combination with a sulfonylurea was not borne out by further analysis, and this combination is widely used. In patients in whom such combinations fail or are contraindicated, pioglitazone or rosiglitazone may be combined with metformin or a sulfonylurea as an alternative to progressing to insulin. Biguanides may also be added to meglitinide therapy. Alpha-glucosidase inhibitors may improve diabetes control when used as an adjunct to sulfonylureas or biguanides. Guar gum may also be used as an adjunct to any of the oral hypoglycaemics to enhance the improvement in blood-glucose control.

Patients with type 2 diabetes who cannot be controlled adequately by oral therapy and diet need insulin either in addition to or in place of oral therapy. Type 2 diabetes is a progressive disease, and about 30% of those on sulfonylureas will be transferred to insulin treatment within 4 years, a change which now tends to be made earlier owing to increasingly strict criteria for glycaemic control (tight control of blood glucose has now been shown to decrease the risk of complications, see Diabetic Complications, below). There is evidence that therapy with insulin and a sulfonylurea is more effective than therapy with insulin alone in type 2 patients.<sup>14</sup> Biguanides and alpha-glucosidase inhibitors may also be combined with insulin. As insulin therapy is associated with more hypoglycaemic episodes and a greater tendency to weight gain it remains reasonable to begin with oral therapy in type 2 diabetes before proceeding to insulin.<sup>15</sup>

Insulin is also substituted for oral treatment to provide cover during periods of severe stress, as in severe infection, trauma, or major surgery. Type 2 patients who become pregnant should also be switched from oral therapy to insulin (see Pregnancy, below).

• **INSULIN THERAPY.** While insulin may not be a necessary part of the treatment of type 2 diabetes, it is essential in the treatment of patients with type 1, since they have little or no endogenous insulin secretory capacity.

The aim of insulin therapy is to achieve the best possible control of blood-glucose concentrations without the risk of the hypoglycaemia that can occur if too fine a degree of control is attempted. Tight control of blood-glucose concentrations can reduce the long-

Table 1. Classification of oral antidiabetics.

Aldose Reductase Inhibitors	Sulfonylureas
Ebálfrestat	Acetohexamide
Sorbinil	Carbutamide
Tolrestat	Chlorpropamide
α-Glucosidase Inhibitors	Glibenclamide
Acarbose	Glibornuride
Miglitol	Gliclazide
Metformin	Glimepiride
Meglitinides	Glipizide
Nateglinide	Glibidone
Repaglinide	Glisentide
Miscellaneous	Glisolamide
Glyburide	Glixocepide
Metformin	Glycycramide
Guan Gum	Tolazamide
Glaglizole	Tolbutamide
	Thiazolidinediones
	Pioglitazone
	Rosiglitazone
	Troglitazone

The symbol † denotes a preparation no longer actively marketed

term complications of diabetes such as retinopathy, nephropathy, and neuropathy (see Diabetic Complications, below) but in some patients (such as the elderly, or those who lack motivation) it may be better merely to alleviate symptoms rather than attempt tight control. Exercise and dietary discipline are necessary to maintain normal sensitivity to insulin.

Insulin may be of beef or pork origin, or it may be human insulin produced by gene technology or by modification of porcine insulin. Human and porcine insulin are less immunogenic than bovine insulin and where possible most newly diagnosed type 1 patients are now given human insulin. Modified insulin analogues such as insulin lispro are now available.

Insulin is available in a range of preparations offering a short, intermediate, or long action on subcutaneous injection. Insulin dosage schedules make use of the varying durations of action, for example by incorporating a short-acting and intermediate-acting insulin into a daily schedule. Most patients with type 1 diabetes require two or three injections of insulin daily, or with intensive regimens even more. A once-daily regimen can be used if the patient is simply to be kept asymptomatic; this may also be successful in patients with type 2 diabetes not satisfactorily controlled by oral antidiabetics.

The various types of insulin may also be given intramuscularly but the subcutaneous route is usually preferred. Soluble insulin can also be given intravenously. Details of insulin administration are given on p.330.

- IMMUNOSUPPRESSION. Many patients with type 1 diabetes experience a temporary improvement in pancreatic beta-cell function soon after initial treatment with insulin. This produces a period of remission known as the honeymoon period during which a small dose of insulin is sufficient to maintain good control. Attempts to prolong the honeymoon period have included tight control immediately following diagnosis and also, given the probable auto-immune nature of the condition, administration of an immunosuppressant.<sup>16-20</sup>

- PANCREATIC TRANSPLANTATION. Transplantation of the whole pancreas in patients with type 1 diabetes poorly controlled by insulin therapy has led to insulin-independence, and has usually been carried out in association with kidney transplantation (see also Other Organs, under Organ and Tissue Transplantation, p.504).<sup>21-23</sup> Transplantation of pancreatic islet cells has been investigated as an alternative.<sup>22-27</sup> However, few patients to date have remained independent of insulin within a year of islet-cell transplantation.<sup>27</sup> Results in animals have suggested that a reasonable degree of glycaemic control may be achievable long term without immunosuppression, using a vascularised 'artificial pancreas' containing allogeneic or even xenogeneic islet cells.<sup>28</sup>

- OTHER DRUG TREATMENTS. Various other drugs have been tried for diabetes mellitus, particularly when conventional therapy has proved unsuccessful. Addition of the amylin analogue pramlintide to insulin therapy has improved glycaemic control in patients with type 1 and type 2 diabetes.<sup>29,30</sup> Other approaches under investigation include inhibition of fatty acid oxidation or the use of  $\beta_3$ -adrenoceptor agonists (selective agonists of  $\beta$ -receptors thought to be associated with lipolysis and thermogenesis) to stimulate energy expenditure.<sup>31</sup>

Mecasermin (insulin-like growth factor I; IGF-1) has been shown to improve metabolic control in patients with type 1 diabetes and insulin resistance (and in some type 2 patients).<sup>32,33</sup> Glucagon-like peptide 1 (GLP-1; insulintropin) improves glycaemic control in type 2 patients.<sup>34</sup> Improvements in insulin sensitivity have also been seen in insulin-resistant type 2 patients treated with haemodialysate of calf blood,<sup>35</sup> while in other studies improved insulin sensitivity was seen following treatment with the vanadium salt, vanadyl sulfate,<sup>36</sup> or chromium supplementation.<sup>37</sup>

- PROPHYLAXIS. Because overt diabetes is the culmination of a prolonged process methods to delay or prevent its development are being investigated, either by modifying risk factors in populations or groups, or by targeting individuals thought to be at high risk. Strategies under consideration for the prevention of type 1 diabetes include avoidance of cows' milk proteins (thought to be a possible environmental trigger) during infancy;<sup>38</sup> administration of free radical scavengers such as nicotinamide;<sup>39</sup> administration of prophylactic insulin<sup>40,41</sup> (or possibly of oral antidiabetics) to allow 'beta cell rest'; encouraging the development of anti-

gen tolerance, for example by the oral administration of antigens such as insulin, glutamic acid decarboxylase, or heat shock protein<sup>41-43</sup> (a similar approach using subcutaneous antigen has apparently extended the honeymoon period in animal studies<sup>44</sup>); or by immunosuppression (see above) or immunomodulation with agents such as BCG vaccine,<sup>45</sup> although some have found the latter ineffective.<sup>46</sup> Preventive strategies for insulin resistance and type 2 diabetes have tended to focus on weight loss and dietary modification. However, prophylactic drug therapy may be possible: a study with troglitazone in nondiabetic obese subjects demonstrated reduced insulin resistance, and improved glucose tolerance in those in whom it was impaired.<sup>47,48</sup>

**Monitoring of therapy.** Monitoring of therapy is an integral part of the management of the diabetic patient. Detection of urinary glucose has generally been superseded by the monitoring of blood glucose (see Glucose Tests, p.1616). For adequate diabetic control, the aim is to reduce fasting blood-glucose concentrations to within the range 3.3 to 5.6 mmol per litre of venous whole blood, and postprandial concentrations to below 10 mmol per litre.<sup>5</sup> Many patients monitor their blood-glucose concentrations regularly at home and this is essential for intensified insulin regimens when tight control is required. The value of self-monitoring of blood-glucose concentrations in type 2 patients is more debatable.<sup>49</sup> Detection of urinary ketones is useful in diabetics prone to ketosis; this is usually performed in clinics. Diabetic clinics also measure the degree of haemoglobin glycosylation (HbA<sub>1</sub> or HbA<sub>1c</sub>) as an indicator of longer term blood-glucose control and hence the risk of complications (see Diabetic Complications, below). More recently the advanced glycation end-product (AGE) of haemoglobin has also been found to be a useful indicator of long-term blood glucose control.<sup>50</sup> Techniques for continuous monitoring of blood glucose are under investigation<sup>51,52</sup> as are noninvasive or minimally invasive methods.<sup>53</sup>

**Pregnancy.** Adverse pregnancy outcomes, including spontaneous abortion and congenital malformation, are more common in diabetic than in nondiabetic women. Improved management of the pregnant diabetic patient, particularly early in pregnancy, lessens the incidence of such events,<sup>54,55</sup> but an increased risk still exists.<sup>56</sup> Diabetic women are advised to plan their pregnancies so that glycaemic control can be improved before conception. Some may need to avoid pregnancy (most commonly because of renal disease),<sup>57</sup> but management has improved sufficiently for this to be rare.<sup>55</sup>

Insulin is the preferred treatment in pregnancy,<sup>58</sup> even in women with type 2 diabetes; patients taking oral antidiabetics should therefore be switched to insulin. Insulin regimens are similar to those in nonpregnant patients, the dose being adjusted according to regular blood-glucose measurements. Insulin requirements may decrease during the first trimester but they increase during the latter two, reaching about twice pre pregnancy requirements at term; they then fall once labour has begun and fall again after delivery.<sup>58</sup>

Pregnant diabetic patients are at risk of nocturnal hypoglycaemia owing to continued fetal glucose consumption while the mother is in a relatively fasting state. They are also prone to diabetic ketoacidosis which must be treated with great urgency because of the high risk of fetal loss.

Gestational diabetes, that is diabetes which develops during pregnancy, may simply be impaired glucose tolerance associated with pregnancy.<sup>59</sup> It is not clear whether treatment of gestational glucose intolerance significantly decreases perinatal mortality or birth-weight.<sup>60</sup> There is, therefore, disagreement about the benefits of universal screening for gestational glucose intolerance.<sup>59-62</sup> Many women with gestational diabetes may be managed by diet alone or, if necessary, also with insulin. Some workers, however, have advocated prophylactic administration of insulin to women who could be managed by diet alone, in view of the metabolic effects of insulin.<sup>63</sup>

**Surgery.** Insulin-dependent diabetics who require surgery may be managed with a continuous intravenous insulin infusion.<sup>64</sup> Insulin is given as normal the night before operation, and switched to either a variable-rate infusion via a syringe pump, together with a 5 or 10% glucose drip (with potassium chloride, provided the patient is not hyperkalaemic), or to a combined insulin-glucose infusion, on the day of operation. (Many anaesthetists prefer insulin and a sodium chloride infusion if blood glucose is already high.<sup>65</sup>) Subsequent conversion back

to subcutaneous insulin should be undertaken before breakfast, giving the first subcutaneous dose 30 minutes before stopping continuous infusion. Non-insulin-dependent patients should have any oral treatment omitted on the day of operation, and may be given insulin by a similar regimen if control is poor or deteriorates as can happen with major surgery.

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5701E Glucolet Endcaps - Super  
October 1996

### GLUCOPHAGE

(Arrow Pharmaceuticals)

**LIFE THREATENING LACTIC ACIDOSIS CAN OCCUR DUE TO ACCUMULATION OF METFORMIN. RISK FACTORS INCLUDE RENAL IMPAIRMENT, OLD AGE AND THE USE OF HIGH DOSES OF METFORMIN ABOVE 2 G PER DAY.**

**Composition:** Metformin hydrochloride.

The chemical name for metformin hydrochloride is 1,1 dimethyl biguanide hydrochloride.

C<sub>4</sub>H<sub>11</sub>N<sub>3</sub>HCl

Molecular weight: 165.6 Cas No: 1115-70-4

**Description:** Metformin hydrochloride is a white, crystalline powder which is odourless or almost odourless and hygroscopic. It is freely soluble in water, slightly soluble in ethanol (96%), and practically insoluble in chloroform and ether.

Glucophage tablets come in two strengths and contain either 500mg or 850mg of metformin hydrochloride. The tablets also contain the following excipients: povidone, magnesium stearate and hypromellose. The tablets are gluten free.

**Pharmacology:** Glucophage is an oral biguanide hypoglycaemic agent. It causes an increased peripheral uptake of glucose by increasing the biological efficiency of available exogenous or endogenous insulin.

The mode of action of metformin may be linked to an increase of insulin sensitivity. It does not stimulate insulin release but does require the presence of insulin to exert its hypoglycaemic effect. Possible mechanisms of action include inhibition of gluconeogenesis in the liver, delay in glucose absorption from the gastrointestinal tract and an increase in peripheral uptake of glucose. Metformin has an antiketogenic activity which is comparable, though somewhat inferior, to insulin itself.

Metformin lowers both basal and post-prandial blood glucose in diabetic patients but does not cause hypoglycaemia in either diabetics or normal individuals.

### Pharmacokinetics:

**Absorption:** After oral administration, metformin hydrochloride is absorbed along the entire gastrointestinal mucosa. Studies using single oral doses of metformin tablets indicate that there is a lack of dose proportionality with increasing doses, which is due to decreased absorption rather than an increase in elimination.

At usual clinical doses and dosing schedules of metformin tablets, steady-state plasma concentrations are reached in 24 to 48 hours and are generally less than 1µg/mL. During controlled clinical trials, maximum metformin plasma levels did not generally exceed 5µg/mL, even at maximum doses.

**Distribution:** Metformin is not bound to plasma proteins.

**Metabolism:** Metformin is excreted unchanged in the urine and does not undergo hepatic metabolism.

**Excretion:** In patients with decreased renal function (based on measured creatinine clearance), the plasma half-life of metformin is prolonged and renal clearance is decreased in proportion to the decrease in creatinine clearance, e.g. if creatinine clearance is 10-30 mL/min, renal clearance is reduced to 20% of normal.

**Indications:** Metformin is indicated in the treatment of type 2 diabetes mellitus not satisfactorily controlled by diet, where the risk of lactic acidosis is minimised by excluding predisposing factors, especially impaired renal, hepatic or cardiovascular function.

Metformin may be used as initial therapy or in sulphonylurea failure, either alone or in combination with a sulphonylurea or as adjuvant therapy in insulin-dependent diabetes.

**Contraindications:** Juvenile diabetes mellitus that is uncomplicated and well regulated on insulin; diabetes mellitus regulated by diet alone; hypersensitivity to biguanides; acute complications of diabetes mellitus such as metabolic acidosis, coma, infection, gangrene, or during or immediately following surgery where insulin is essential.

Glucophage should be temporarily withheld in patients undergoing radiological studies involving intravascular administration of iodinated contrast materials, because use of such products may result in acute alteration of renal function.

**Risk of lactic acidosis:** Because of the danger of lactic acidosis, metformin should not be used in the presence of the following conditions: diminished renal function; cardiovascular disease (e.g. coronary insufficiency, myocardial infarction and hypertension); conditions which may be associated with tissue hypoxia (e.g. gangrene, circulatory shock, acute significant blood loss); pulmonary embolism; severe hepatic dysfunction; pancreatitis; excessive alcohol intake; concomitant use of diuretics.

**Precautions:** Lactic acidosis is a rare but serious metabolic complication which can occur due to metformin accumulation during treatment with

Glucophage. When it occurs, it is fatal in approximately 50% of cases. Lactic acidosis is a medical emergency and must be treated in hospital immediately. The risk of lactic acidosis increases with the degree of renal dysfunction and the patient's age. Reported cases have occurred primarily in diabetic patients with significant renal insufficiency, often in the setting of multiple concomitant medical/surgical problems and multiple concomitant medications.

The reported incidence of lactic acidosis in patients receiving metformin is very low (approximately 0.03 cases per 1,000 patient years, with approximately 0.015 fatal cases per 1,000 patient years). The onset is often subtle and accompanied by nonspecific symptoms such as malaise, myalgia, respiratory distress, increasing somnolence and nonspecific abdominal distress. Lactic acidosis may also occur in association with a number of pathophysiological conditions, including diabetes mellitus, and when there is significant tissue hypoperfusion and hypoxaemia. Lactic acidosis is characterised by acidosis (decreased blood pH), elevated lactate levels with increased lactate/pyruvate ratio and electrolyte disturbances with an increased anion gap.

When metformin is implicated as the cause of lactic acidosis, metformin plasma levels greater than 5µg/mL are generally found (See Pharmacokinetics). Underlying renal disease, or a deterioration in renal function, result in reduced clearance of metformin and drug accumulation and are therefore major risk factors in lactic acidosis. The risk of lactic acidosis may therefore be significantly decreased by regular monitoring of renal function in patients taking metformin and by the use of the minimum effective dose of metformin. In addition, metformin therapy should be temporarily stopped in the presence of any condition associated with hypoxaemia or dehydration, in patients suffering from serious infections or trauma (particularly if gastrointestinal disturbances are noted or acidosis is suspected) and in those undergoing surgery.

Radiological studies involving the use of intravascular iodinated contrast materials (for example intravenous urogram, intravenous cholangiography, angiography, any computed tomography scans with intravascular contrast materials) can lead to acute alteration of renal function and have been associated with lactic acidosis in patients receiving metformin. Therefore, in patients in whom any such study is planned, Glucophage should be discontinued at the time of, or prior to, the procedure, and reinstated only after renal function has been re-evaluated and found to be normal.

Alcohol is known to potentiate the effect of metformin on lactate metabolism. Patients should therefore be warned against excessive alcohol intake, acute or chronic, while taking metformin.

Periodic assessment of renal, hepatic and cardiovascular function is recommended during prolonged periods of treatment with metformin. Patients receiving continuous metformin therapy should have an annual estimation of vitamin B12 levels because of reports of decreased vitamin B12 absorption.

**Carcinogenicity/Mutagenicity:** Long term carcinogenicity studies have been performed in rats (dosing duration of 104 weeks) and mice (dosing duration of 91 weeks) at doses up to and including 900mg/kg/day and 1500mg/kg/day, respectively. These doses are both approximately two to three times the recommended human daily dose on a body surface area basis. No evidence of carcinogenicity with metformin was found in either male or female mice. Similarly, there was no tumourigenic potential observed with metformin in male rats. However, an increased incidence of benign stromal uterine polyps was seen in female rats treated with 900mg/kg/day.

No evidence of a mutagenic potential of metformin was found in the Ames test (*S. typhimurium*), gene mutation test (mouse lymphoma cells), chromosomal aberrations test (human lymphocytes), or in vivo micronuclei test (mouse bone marrow).

Fertility of male or female rats was unaffected by metformin administration at doses up to 600mg/kg/day, or approximately twice the maximum recommended human daily dose on a body surface area basis.

**Use in Pregnancy:** Category C. Since it is important to achieve strict normoglycaemia during pregnancy, metformin should be replaced by insulin. Metformin was not teratogenic in rats and rabbits at doses up to 600mg/kg/day, or about two times the maximum recommended human daily dose on a body surface area basis. Determination of fetal concentrations demonstrated a partial placental barrier to metformin. Because animal reproduction studies are not always predictive of human response, any decision to use this drug should be balanced against the benefits and risks. The safety of metformin in pregnant women has not been established.

Because recent information suggests that abnormal blood glucose levels during pregnancy are associated with a higher incidence of congenital abnormalities, there is a consensus among experts that insulin be used during pregnancy to maintain blood glucose levels as close to normal as possible.

# June 2003 MIMS Annual

## Foreword

This edition of MIMS Annual takes this publication, which is the byword for accurate, reliable, comprehensive and independent drug information, into its twenty-eighth year of meeting the ever-growing demand by healthcare professionals for decision support information on which they can depend to assist in the management of their patients. The fact that MIMS Annual has grown almost exponentially over time is testament to the growth in this demand and reflects the increasing number and complexity of new pharmaceutical products being introduced to the Australian healthcare market. Specifically in this 2003 edition there are 222 products flagged in the database as NEW listings and 651 products flagged as having revised information, all contained within 1,716 pages – the largest MIMS Annual yet.

As we move further into the electronic era the demand for an interactive presentation of decision support information continues to grow. The more complex and detailed the information, the greater the need for strong search tools to facilitate quick access. The MIMS Annual database is now available in CD and on-line formats and in 2003 the eMIMS CD offers the user the opportunity to search full or brief prescribing information by any combination of up to 12 criteria, with full colour identification photographs at the click of a mouse.

eMIMS now includes a new stand-alone drug interactions module, and fully searchable electronic versions of MIMS Disease Index and MIMS Companion - unique Australian references - all hyperlinked to the approved prescribing information by appropriate keywords. This CD release also offers over 1,600 Consumer Medicine Information (CMI) documents which can be printed out, when appropriate, for patients. In addition on the 2003 eMIMS you can access MIMS Assist, a comprehensive listing of almost 1,200 special advisory and consulting services for patient counselling and lifestyle management in acute and chronic situations.

The MIMS Annual in print format and eMIMS are designed to complement each other as references, depending on the situation. In today's litigation conscious environment prescribers, and healthcare professionals in general, need to be confident that the information used as decision support, in both electronic and print formats, is reliable, accurate, from a trusted source AND reflects the current APPROVED information. With over 40 years of experience in providing drug information to Australia's healthcare professionals, MIMS meets all these requirements. As a member of the MediMedia International group MIMS is part of the world's largest global healthcare information company. The benefit of having access to the latest in content and technology resources worldwide will flow on to our subscribers as we release new decision support tools over the next year.

We are sure that you will find the 2003 edition of Australia's most widely used drug reference of even more value in your practice than before. As always we welcome your feedback so we can continue to make MIMS Annual and its companion products as useful as possible.

For further information on MIMS Annual and any other MIMS products please contact the publisher, MIMS Australia.

Yours sincerely,

C. R. Wills BPharm MPS MRPharmS

Managing Director & Publisher

Product monographs in MIMS Annual represent Therapeutic Goods Administration (TGA) approved product information, which is the result of years of research and development by the sponsor company and of painstaking evaluation and review by the Drug Safety and Evaluation Branch of the TGA. If the information in MIMS Annual appears at times to differ from other published product information, it is due to our editorial process of standardising headings, format and terminology. Always with the users' needs in mind, this editing results in a reference book where the desired information can be located under consistent headings, which appear in the same sequence in each monograph. It is this rigorous attention to detail that has made MIMS Annual a standard reference for approved prescribing information throughout Australia.

**It is however important to remember that the selection of therapy based on information contained in MIMS Annual should always be overlaid against the profile of the individual patient. Clinical decisions are the responsibility of the attending physician and where any doubt exists regarding the appropriateness or not of any therapy, referral to a relevant specialist and/or the specific pharmaceutical manufacturer is recommended. There will inevitably be clinical situations which will not be fully covered by the approved information. In these circumstances it is strongly recommended that further advice be sought before prescribing.**

## 6e Hypoglycaemic agents

### 6e Hypoglycaemic agents

#### Actos

Revised Entry this Edition

Eli Lilly Australia Pty Ltd

**Composition** Active: Pioglitazone hydrochloride.

Inactive: Lactose, hydroxypropylcellulose, carmellose calcium and magnesium stearate.

**Description** Chemical name:  $(\pm)$ -5-[4-[2-(5-ethyl-2-pyridinyl)ethoxy]phenyl]methyl]-2,4-thiazolidinedione hydrochloride. Molecular formula:  $C_{19}H_{22}N_2O_3S \cdot HCl$ . MW: 392.90. CAS: 112529-15-4 (pioglitazone HCl); 111025-46-8 (pioglitazone free base).

Pioglitazone hydrochloride is an odourless, white crystalline powder that is soluble in N,N-dimethylformamide, slightly soluble in anhydrous ethanol, very slightly soluble in acetone and acetonitrile, practically insoluble in water and insoluble in ether.

**Actions Pharmacology** Actos is an oral antidiabetic agent that acts primarily by decreasing insulin resistance. Pharmacological studies indicate that Actos improves sensitivity to insulin in muscle and adipose tissue and inhibits hepatic gluconeogenesis. Actos improves glycaemic control while reducing circulating insulin levels.

Fasting and postprandial glycaemic control are improved in patients with type 2 diabetes mellitus. The decreased insulin resistance produced by Actos results in lower blood glucose concentrations, lower plasma insulin levels and lower HbA<sub>1c</sub> values. Mode of action. Actos is a thiazolidinedione antidiabetic agent that depends on the presence of insulin for its unique mechanism of action. Actos decreases insulin resistance in the periphery and in the liver resulting in increased insulin dependent glucose disposal and decreased hepatic glucose output. Unlike sulfonylureas, pioglitazone is not an insulin secretagogue. Pioglitazone is a potent and highly selective agonist for peroxisome proliferator activated receptor gamma (PPAR $\gamma$ ). PPAR receptors are found in tissues important for insulin action such as adipose tissue, skeletal muscle and liver. Activation of PPAR $\gamma$  nuclear receptors modulates the transcription of a number of insulin responsive genes involved in the control of glucose and lipid metabolism.

In animal models of diabetes, pioglitazone reduces the hyperglycaemia, hyperinsulinaemia and hypertriglyceridaemia characteristic of insulin resistant states such as type 2 diabetes. The metabolic changes produced by pioglitazone result in increased responsiveness of insulin dependent tissues and are observed in numerous animal models of insulin resistance.

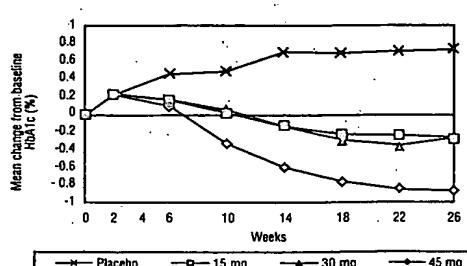
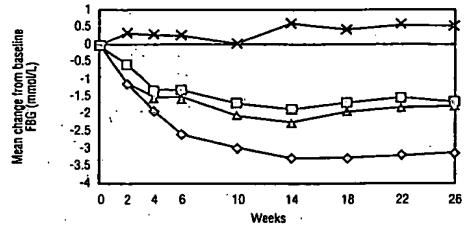
Since pioglitazone enhances the effects of circulating insulin (by decreasing insulin resistance), it does not lower blood glucose in animal models that lack endogenous insulin.

**Clinical trials.** Clinical studies demonstrate that Actos improves insulin sensitivity in insulin resistant patients. Actos enhances cellular responsiveness to insulin, increases insulin dependent glucose disposal, improves hepatic sensitivity to glucose and thus improves dysfunctional glucose homeostasis.

**Monotherapy.** Three randomised, double blind, placebo controlled trials of 16 to 26 weeks were conducted to study the use of Actos as monotherapy in patients with type 2 diabetes. These studies examined Actos doses from 7.5 to 45 mg/day in 865 patients.

In a 26 week dose ranging study, 408 patients with type 2 diabetes were randomised to receive Actos 7.5, 15, 30 or 45 mg, or placebo. Compared with placebo, treatment with Actos 15 to 45 mg resulted in significant improvements in HbA<sub>1c</sub> and fasting blood glucose (FBG) (see Figure 1).

**Actos**  
Mean change from baseline for FBG and HbA<sub>1c</sub> in a 26 week placebo controlled dose ranging study



The study population included patients not previously treated with antidiabetic medication (naive 31%) and patients who were receiving antidiabetic medication at the time of study enrolment (previously

treated 69%). The data for the naive and previously treated patient subsets are shown in Table 1. This run-in period was associated with little change in HbA<sub>1c</sub> and FBG values from screening to baseline for the naive patients. However, for the previously treated group,退出 from previous antidiabetic medication resulted in deterioration in glycaemic control and increases in HbA<sub>1c</sub> and FBG. With Actos, over most patients in the previously treated group had a decrease from baseline in HbA<sub>1c</sub> and FBG, in many cases the values did not return to screening levels by the end of the study. The study design did not permit the evaluation of patients who switched directly to Actos from another antidiabetic agent.

**Actos**  
Table 1  
Glycaemic parameters in a 26 week placebo controlled dose ranging study

	Actos 15 mg once daily	Placebo
Naive to therapy		
HbA <sub>1c</sub> (%)	n = 25	n = 26
Screening (mean)	9.3	10.0
Baseline (mean)	9.0	9.9
Change from baseline (adjusted mean*)	0.6	-0.8
Difference from placebo (adjusted mean*)	-1.4	-1.4
FBG (mmol/L)	n = 25	n = 26
Screening (mean)	12.39	13.61
Baseline (mean)	12.72	13.94
Change from baseline (adjusted mean*)	0.89	-2.06
Difference from placebo (adjusted mean*)	-2.89	-2.89
Previously treated		
HbA <sub>1c</sub> (%)	n = 54	n = 53
Screening (mean)	9.3	9.0
Baseline (mean)	10.4	10.4
Change from baseline (adjusted mean*)	0.8	-1.0
Difference from placebo (adjusted mean*)	-1.0	-1.0
FBG (mmol/L)	n = 54	n = 53
Screening (mean)	12.33	11.61
Baseline (mean)	15.83	15.28
Change from baseline (adjusted mean*)	0.22	-1.78
Difference from placebo (adjusted mean*)	-2.00	-2.00

\* Adjusted for baseline, pooled centre

Pioglitazone has been shown to reduce total plasma triglycerides, free fatty acids and to increase HDL cholesterol levels. LDL cholesterol levels remain unchanged. In a 26 week, placebo controlled dose ranging study, mean triglyceride levels decreased in the 15, 30 and 45 mg dose groups compared to a mean increase in the placebo group. Mean HDL levels increased to a greater extent in the Actos treated patients than in the placebo treated patients. There were no consistent differences for LDL and total cholesterol between treated patients compared with placebo (see Table 2).

**Actos**  
Table 2  
Lipids in a 26 week placebo controlled dose ranging study

	Actos 15 mg once daily	Placebo
Triglycerides (mmol/L)	n = 79	n = 79
Baseline (mean)	2.97	3.20
Percent change from baseline (mean)	4.8%	-9.3%
HDL cholesterol (mmol/L)	n = 79	n = 79
Baseline (mean)	1.08	1.08
Percent change from baseline (mean)	8.1%	14.1%
LDL cholesterol (mmol/L)	n = 65	n = 63
Baseline (mean)	3.59	3.41
Percent change from baseline (mean)	4.8%	7.2%
Total cholesterol (mmol/L)	n = 79	n = 79
Baseline (mean)	5.81	5.60
Percent change from baseline (mean)	4.4%	4.5%

In a separate 24 week study, 260 patients with type 2 diabetes were randomised to one of two forced titration Actos treatment groups (n=130) or a mock titration placebo arm (n=130). In the treatment group, patients received an initial dose of 7.5 mg once daily. After four weeks, the dose was increased to 15 mg once daily and after another four weeks, the dose was increased to 30 mg once daily for the remainder of the study (16 weeks). In the placebo group, patients received an initial dose of 15 mg once daily and were titrated to 30 mg once daily and 45 mg once daily in a similar manner. Treatment with Actos, as described, produced statistically significant improvements in HbA<sub>1c</sub> and FBG at endpoint compared with placebo (see Table 3).

**Actos**  
Table 3  
Glycaemic parameters in a 24 week placebo controlled forced titration study

	Actos	Placebo
Total population		
HbA <sub>1c</sub> (%)	n = 83	n = 83
Baseline (mean)	10.8	10.8
Change from baseline (adjusted mean**)	0.9	0.9
Difference from placebo (adjusted mean**)	-0.1	-0.1
FBG (mmol/L)	n = 78	n = 78
Baseline (mean)	15.50	15.50
Change from baseline (adjusted mean**)	1.00	1.00

\* Final dose in forced titration  
\*\* Adjusted for baseline, pooled centre, and pooled centre by treatment group

p < 0.05 versus placebo

For patients who had not been previously treated with antidiabetic medication (24%), mean values at screening were 10.7% HbA<sub>1c</sub> and 13.22 mmol/L for FBG. At baseline, mean HbA<sub>1c</sub> was 11.0% and mean FBG was 13.55 mmol/L. Compared with placebo, treatment with Actos titrated to a final dose of 30 and 45 mg resulted in reductions from baseline in mean HbA<sub>1c</sub> of 2.3 and 2.6%, and mean FBG of 5.26 and 5.88 mmol/L, respectively. For patients who had been previously treated with antidiabetic medication (76%), this mean reduction in HbA<sub>1c</sub> was discontinued at screening. Mean values at screening were 10.7% HbA<sub>1c</sub> and 12 mmol/L for FBG. At baseline, mean HbA<sub>1c</sub> was 11.0% and mean FBG was 16.11 mmol/L. Compared with placebo, treatment with Actos titrated to a final dose of 30 and 45 mg resulted in reductions from baseline in mean HbA<sub>1c</sub> of 1.3 and 1.4% and mean FBG of 3.06 and 3.33 mmol/L, respectively. For many previously treated patients, HbA<sub>1c</sub> and FBG had not returned to screening levels by the end of the study.

RAIBRS Annual 2002

## 6e Hypoglycaemic agents

Table 5 12 week cumulative incidence of adverse events at > 2% of Actos treated patients		
Adverse event	Placebo (n = 431)	Pioglitazone (n = 862)
Upper respiratory tract infection	7.2	8.7
Headache	6.5	7.0
Sinusitis	2.9	3.6
Myalgia	2.3	3.2
Oedema	0.6	3.2
Back pain	2.3	3.1
Urinary tract infection	1.6	2.7
Pharyngitis	0.3	2.7
Tooth disorder	1.5	2.6
Fatigue	2.4	2.5
Accidental injury	1.5	2.2
Cramps (legs)	1.1	2.1
Vision abnormal	1.4	2.1

Although pioglitazone does not change the safety profile of sulfonylureas and insulin, the combination may increase the risk of developing hypoglycaemic symptoms. In combination therapy studies, oedema was reported for 7.2% of patients treated with Actos and sulfonylureas compared to 2.1% of patients on sulfonylureas alone. In combination therapy studies with metformin, oedema was reported in 6.0% of patients on combination therapy compared to 2.5% of patients on metformin alone. In combination therapy studies with insulin, oedema was reported in 15.3% of patients on combination therapy compared to 7.0% of patients on insulin alone (see Precautions, Oedema). Most of these events were considered mild or moderate in intensity.

In all clinical trials, weight increased proportionately as the HbA<sub>1c</sub> decreased, suggesting that weight gain was associated with improved glycaemic control. Occasional transient increases in creatinine phosphokinase were noticed in patients taking pioglitazone.

**Cardiovascular system.** In clinical studies, the incidence of serious cardiac adverse events has not been shown to be increased in patients treated with pioglitazone either when used as monotherapy or in combination with metformin or sulfonylurea. In insulin combination studies a small number of patients with previously existing cardiac disease developed congestive heart failure when treated with pioglitazone.

**Laboratory test abnormalities. Haematological.** Actos may cause decreases in haemoglobin and hematocrit. Across all clinical studies, mean haemoglobin values declined by 2 to 4% in patients treated with Actos. These changes generally occurred within the first 4 to 12 weeks of therapy and remained relatively stable thereafter. These changes may be related to increased plasma volume associated with Actos therapy and have not been associated with any significant haematological clinical effects.

**Serum transaminase levels.** During placebo controlled clinical trials in the US, a total of 4 of 1,526 (0.26%) patients treated with Actos and 2 of 793 (0.25%) placebo treated patients had ALT values ≥ three times the upper limit of normal. During all clinical studies in the US, 11 of 2,561 (0.43%) patients treated with Actos had ALT values ≥ three times the upper limit of normal. All patients with follow-up values had reversible elevations in ALT. In the population of patients treated with Actos, mean values for bilirubin, AST, ALT, alkaline phosphatase and GGT were decreased at the final visit compared with baseline. Fewer than 0.12% of patients treated with Actos were withdrawn from clinical trials in the US due to abnormal liver function tests. In preapproval clinical trials, there were no cases of idiosyncratic drug reactions leading to hepatic failure (see Precautions, Use in hepatic impairment).

**CPK levels.** During required laboratory testing in clinical trials, sporadic, transient elevations in creatine phosphokinase levels (CPK) were observed. A single, isolated elevation to greater than ten times the upper limit of normal (values of 2,150 to 8,610) was noted in seven patients. Five of these patients continued to receive Actos and the other two patients had completed receiving study medication at the time of the elevated value. These elevations resolved without any apparent clinical sequelae. The relationship of these events to Actos therapy is unknown.

**Adverse events identified from spontaneous postmarketing surveillance. Cardiovascular system.** Cardiac failure (very rare). In postmarketing experience with pioglitazone, congestive heart failure has been reported in patients both with and without pre-existing cardiac disease.

**Digestive system.** Hepatocellular dysfunction. In postmarketing experience with pioglitazone, reports of hepatitis and hepatic enzyme elevations to three or more times the upper limit of normal have been received. Very rarely, these have involved hepatic failure with and without fatal outcome, although causality has not been established.

**Dosage and Administration** Actos should be taken once daily with or without food.

**Monotherapy.** The recommended dosage of Actos is 15 or 30 mg once daily, increasing after four weeks, if greater therapeutic effect is needed, to 45 mg once daily.

**Combination therapy.** The recommended dose of Actos is 30 mg once daily in combination with sulfonylureas, insulin or metformin. It may be possible to achieve metabolic control at a reduced dose of the sulfonylurea, insulin or metformin. If there is a particular risk of hypoglycaemia, pioglitazone can be introduced at a dose of 15 mg. For patients already on insulin, pioglitazone should be introduced at a dose of 15 mg once daily. Dosage can then be increased cautiously.

**Maximum recommended dose.** The dose should not exceed 45 mg/day since doses higher than 45 mg/day have not been studied in clinical trials.

**Patients with renal insufficiency.** Dose adjustment in patients with renal insufficiency is not recommended (see Actions, Pharmacokinetics). No information is available for patients on dialysis therefore Actos should not be used in such patients.

**Patients with hepatic impairment.** The intrinsic clearance of pioglitazone may be reduced in patients with hepatic disease. Dosage should start at 15 mg and be increased cautiously. Actos therapy should not be initiated in patients with increased baseline liver enzyme levels (ALT > 2.5 times the upper limit of normal).

**Overdosage.** During clinical trials, one case of overdose with Actos was reported. A patient took 120 mg/day for four days, then

180 mg/day for seven days. The patient did not report any clinical symptoms.

Hypoglycaemia would not be expected with Actos alone but may occur in combination with sulfonylureas or insulin. Symptomatic and general supportive measures should be taken in case of overdose.

**Presentation** Tablets, 15 mg, 30 mg, 45 mg; 7's (sample pack), 28's.

**See Product Identification Guide.** Actos 15 mg tablet (grid 16, 742);

Actos 30 mg tablet (grid 17, 758); Actos 45 mg tablet (grid 18, 765).

**Poisons Schedule S4.**

**TGA approval/last amendment:** 27/05/2002.

### Amaryl

Aventis Pharma Pty Limited

**Composition** Active: Glimepiride.

Inactive: Lactose, sodium starch glycolate, povidone, microcrystalline cellulose and magnesium stearate. Additionally, each strength contains the following identifying pigments: 1 mg tablet: iron oxide red; 2 mg tablet: iron oxide yellow and indigo carmine lake; 3 mg tablet: iron oxide yellow; 4 mg tablet: indigo carmine lake.

**Description** Glimepiride is a member of the sulfonylurea group of oral antidiabetic agents. Chemical name: trans-1-[4-(2-(3-ethyl-4-methyl-2-oxo-3-pyrolin-1-carboxamido)ethyl)-phenylsulfonyl]-3-(4-methylcyclohexyl) urea. Molecular formula:  $C_{24}H_{34}N_4O_5S$ , MW: 491. CAS: 93479-97-1.

Glimepiride is a white odourless, crystalline powder, practically insoluble in methanol and water, slightly soluble in ethanol and sparingly soluble in methylene chloride.

**Actions** Sulfonylurea antidiabetic agent.

**Pharmacology** Glimepiride is a sulfonylurea antidiabetic agent which decreases blood glucose concentrations. The primary mechanism of action of glimepiride appears to be dependent on stimulating the release of insulin from functioning pancreatic β cells. Glimepiride acts in concert with glucose by improving the sensitivity of β cells to physiological glucose stimulus, resulting in insulin secretion in the rhythm of meals. In addition, extrapancreatic effects (e.g. reduction of basal hepatic glucose production and increased peripheral tissue sensitivity to insulin and glucose uptake) may also play a limited role in the activity of glimepiride.

In nonfasting diabetic patients, the hypoglycaemic action of a single dose of glimepiride persists for 24 hours.

Evidence from *in vitro* and animal studies suggests that there is lower glucagon secretion with glimepiride than glibenclamide and this may give rise to a prolonged reduction of blood glucose levels without increased plasma insulin levels. The clinical significance of these findings is yet to be clarified. A long-term, randomised, placebo controlled clinical trial demonstrated that Amaryl therapy improves postprandial insulin/C-peptide responses and overall glycaemic control without producing clinically meaningful increases in fasting insulin/C-peptide levels.

The efficacy of Amaryl is not affected by age, gender or weight. Amaryl therapy is effective in controlling blood glucose without deleterious changes in the plasma lipoprotein profile of patients. The physiological response to acute exercise (i.e. reduction of insulin secretion) is still present during glimepiride therapy.

**Clinical trials.** A placebo controlled study using fixed daily doses of 1, 4 and 8 mg glimepiride found that all three doses were effective at reducing blood glucose levels. However, there was no significant difference in the reduction in fasting plasma glucose (FPG) between the 4 and 8 mg doses at any timepoint throughout the study.

In another placebo controlled dose ranging study of glimepiride (1, 2, 3, 4, 6 and 8 mg/day), the majority of patients were controlled in the dose range of 1 to 4 mg/day. There was only a very small difference in the reduction in median FPG levels between the 4 and 8 mg doses (-3.08 mmol/L versus -3.16 mmol/L). The greatest change of -3.27 mmol/L was seen with the 2 mg dose. This supports the results of the aforementioned clinical study.

Two large multicentre studies involving approximately 1,900 patients were conducted to examine the dose-response effect of glimepiride on blood glucose and HbA<sub>1c</sub> levels. In both these studies, a large proportion of patients achieved an FPG level below 8.32 mmol/L at the 1 mg/day dose, with a further 10% of patients achieving this level at the 2 mg/day dose. Some patients benefited by an increase in dose to 4 mg/day, but only a few patients, mainly those with very high baseline FPG levels, required higher doses. Based on the results of these studies, the World Health Organization (WHO) has set the defined daily dose (DDD) of glimepiride to be 2 mg.

An additional 161 patient, randomised, double blind crossover study, including four weeks active treatment each with 3 mg bd (twice daily) or 6 mg daily of glimepiride, indicated that some patients may have improved results when glimepiride is given twice daily. However, for the majority of patients, once daily dosing provided adequate control. It is important to note that the treatment period in this study was only four weeks and, as such, the long-term safety benefit of twice daily dosing has not been established.

**Pharmacokinetics.** The pharmacokinetics of glimepiride are similar in males and females and also in young and elderly (> 65 years) patients. Intraindividual variability is low.

**Absorption.** Glimepiride is completely absorbed after oral administration.

The peak serum concentration (Cmax) is reached in about 2.5 hours. There is a linear relationship between dose and both Cmax and AUC (area under the plasma concentration-time curve). Food does not significantly affect the rate or extent of absorption of glimepiride.

**Distribution.** After intravenous dosing in normal subjects, the volume of distribution was 8.8 L (113 mL/kg) and the total body clearance was 48 mL/minute. Protein binding was greater than 99%.

Glimepiride is likely to be only minimally removed by haemodialysis due to its high protein binding.

Multiple dose studies with glimepiride in diabetic patients demonstrated plasma concentration-time curves similar to single dose studies, indicating that there is no accumulation of drug in tissue depots.

**Metabolism.** The elimination half-life of glimepiride at steady state is about five to eight hours after oral administration. However, results of a pharmacokinetic study on noninsulin dependent diabetes mellitus (NIDDM) patients indicated that higher doses may be associated with a longer half-life.

Glimepiride is completely metabolised by oxidative biotransformation. The major metabolites are the cyclohexyl hydroxyl derivative (M1) and the carboxyl derivative (M2). *In vitro* studies indicate that cytochrome P450 2C9 is the principal enzyme involved in the biotransformation of glimepiride to M1. M1 has been found to account for about 40% of the pharmacological activity of glimepiride excreted via the urine and also by further metabolism to M2. M2 is excreted via several cytosolic enzymes. M1 has a terminal elimination half-life of three to six hours after an oral dose. The formation of M1 is proportional to a dose of glimepiride 16 mg. The kinetics of M2 have not been fully elucidated due to low plasma levels. Its terminal elimination half-life after an oral dose is about five to six hours.

Following an oral dose of glimepiride, 35% of the dose is excreted in faeces and 58% in urine.

**Renal Insufficiency.** In patients with renal impairment, there is a tendency for glimepiride clearance to increase and to result in serum concentrations to decrease, most probably because of the more rapid elimination because of lower protein binding. The elimination of the two metabolites was impaired. Overall, glimepiride appears to be no increased risk of accumulation in these patients.

A multiple dose titration study in patients with mild to moderate renal impairment showed that approximately 44% had adequate reductions in their glucose levels with a dosage regimen of 1 mg/day. The results from this study suggest that a starting dose of 1 mg/day may be given to an NIDDM patient with renal disease and that this may be titrated based on fasting blood glucose levels (see Dosage and Administration).

**Hepatic failure.** The effects of hepatic failure on the pharmacokinetics of glimepiride have not been systematically examined.

**Indications** Adjunct to diet, exercise and weight loss in the treatment of diabetes mellitus in patients with noninsulin dependent diabetes (type II diabetes).

**Contraindications** Hypersensitivity to glimepiride, sulfonylureas, other sulphonamides or any excipient.

**Severe Impairment of renal function.**

**Dialysis patients.**

**Severe hepatic dysfunction.**

**Pregnancy (see Precautions, Use in pregnancy).**

**Lactation (see Precautions, Use in lactation).**

**Amaryl** is not suitable for the treatment of insulin dependent diabetes mellitus (e.g. for the treatment of patients with ketoacidosis), nor for the treatment of diabetic ketosis, nor for the treatment of diabetic precoma or coma.

**Precautions** Patients receiving glimepiride should be monitored with regular clinical and laboratory evaluations including serum glucose determinations, to determine the appropriate dose and to detect primary failure (inadequate low blood glucose concentration at the maximum recommended dose) and secondary failure (loss of control of blood glucose levels following an initial period of effectiveness) to the drug. Haemoglobin measurements may also be useful to monitor the patient's response to glimepiride therapy.

Some improvement in glucose tolerance may take up to four weeks of treatment with glimepiride. The clinician should check within the first four to eight weeks and thereafter periodically thereafter to ascertain whether it is possible to reduce the dose. The treatment of diabetes requires regular monitoring. Hypoglycaemic reactions may be impaired due to hypoglycaemic drugs, especially when beginning or after altering treatment, if the patient is not taken regularly. This may, for example, affect the ability to drive a vehicle or to operate machinery.

In cases of unusual stress (e.g. emergency of electrical shock, infection), a temporary change to insulin may be necessary to maintain good metabolic control.

Amaryl tablets must not be used beyond the expiry date on the pack and must be stored out of the reach of children.

**Hypoglycaemic reactions.** Hypoglycaemia is a common side effect of treatment with any sulfonylurea, particularly at the onset of treatment or when dosage is increased.

Debilitated patients, malnourished patients and patients with pituitary, renal or hepatic insufficiency are particularly susceptible to the hypoglycaemic action of sulfonylureas and should be closely monitored. The dosage of glimepiride should be adjusted in these patients.

Hepatic insufficiency may cause increased serum glucose levels. Glimepiride and may diminish glucoregulatory function and increase the risk of severe hypoglycaemic reactions.

Alcohol ingestion, severe or prolonged exercise, low carbohydrate intake or use of more than one antidiabetic agent may contribute to the development of hypoglycaemic reactions.

Hypoglycaemia may be difficult to recognise in patients who are taking β-adrenergic blocking drugs, central nervous system pathogenic agents.

Patients and responsible family members should be informed of the signs and symptoms of hyperglycaemia (e.g. frequent micturition, dry skin) and hypoglycaemia (e.g. sweating, tremor, restlessness, irritability, drowsiness, disturbed sleep or transient neurological disorders). Action to be taken if either event should occur should be explained.

The potential for primary and secondary failure should be explained.

Hypoglycaemia can almost always be promptly relieved by oral intake of carbohydrates (glucose or sugar). It is important to take sulfonylureas that, despite initial successful countermeasures, may cause hypoglycaemia. Patients must therefore remain under observation. Severe hypoglycaemia requires immediate treatment.

monitoring-up by a doctor and, in some circumstances, in-patient medical care.

**Carcinogenesis, mutagenesis, impairment of fertility.** A standard battery of laboratory tests did not reveal any genotoxic or mutagenic potential for glimepiride. In a two year carcinogenicity study in rats receiving glimepiride in the diet up to 813 mg/kg/day, there was no increase in the incidence of pancreatic islet cell hyperplasia and tumour adenomas; these are regarded to be the result of chronic stimulation of the pancreatic  $\beta$  cells. In a 30 month carcinogenicity study in rats receiving glimepiride in the diet up to 345 mg/kg/day, there was an increased incidence of pancreatic islet cell adenomas, however these were considered incidental as there was no dose dependency in either sex. There were no malignant tumours in rats receiving glimepiride.

**Pregnancy.** (Category C) It is important to achieve strict glycaemic control during pregnancy. Oral hypoglycaemic agents must be replaced by insulin. The sulfonylureas may enter the fetal circulation and cause neonatal hypoglycaemia. In rats, dietary doses at high doses (approximately 82 mg/kg) during gestation caused limb deformations. In rabbits, effects on pregnancy were minimised by increased incidences of abortions/total resorptions and stillbirths. Similar fetal wastage was not seen in rats although the finding of anophthalmia in a proportion of fetuses may be related to the treatment related effect as eye malformations were also seen in the rabbit study. Adverse pregnancy outcomes in the rat and rabbit are probably due to the pharmacodynamic effects of insulin. Excessive doses and are not substance specific. Glimepiride had no recognisable effects on the rearing, physical growth, functional and learning behaviour, memory or fertility of the offspring.

**Lactation.** Studies in rats showed that glimepiride is excreted in milk. High doses caused hypoglycaemia in sucking young rats. After administration of glimepiride (120 to 206 mg/kg) during lactation caused limb deformations in adolescent pups from day 4 of lactation onwards. To prevent possible ingestion of glimepiride with breastmilk, breastfeeding mothers must either be changed over to insulin or cease breastfeeding.

**Children.** The safety and efficacy of glimepiride in children has not been established. Glimepiride is not recommended for use in children.

**Interactions.** Based on experience with glimepiride and known interactions for other sulfonylureas, the following interactions must be considered:

**Drugs which potentiate the hypoglycaemic action of glimepiride.** Drugs which potentiate the hypoglycaemic action of glimepiride include ACE inhibitors, alginic acid, anabolic steroids, chloramphenicol, clonazepam derivatives, fenfluramine, monoamine oxidase inhibitors (MAOIs), miconazole, oxpentifyline, oxyphenbutazone, phenothiazines, propranolol, probenecid, salicylates, sulfapyrazone, sulfonylureas, tetracyclines.

**Drugs which may attenuate the hypoglycaemic action of Amaryl.** Drugs which may attenuate the hypoglycaemic action of Amaryl include barbiturates, calcium channel blockers, corticosteroids, diazepam, diuretics, glucagon, isoniazid, laxatives (prolonged), isoproterenol, methocarbamol, nonsteroidal anti-inflammatories, sympathomimetic agents, thyroid hormones, tricyclic antidepressants,  $\beta$ -blockers, clonidine and reserpine may reduce the potentiation or weakening of the blood glucose lowering effect of glimepiride.

**Drugs which may mask the warning symptoms of a hypoglycaemic attack.**

**Alcohol.** Alcohol intake may either potentiate or attenuate the hypoglycaemic action of Amaryl in an unpredictable fashion.

**Enzyme inducers.** Enzyme inducers may be potentiated or weakened. Clinical studies indicate that glimepiride is metabolised via the enzyme CYP2C9. Therefore, interactions with drugs which are metabolised via CYP2C9 inhibit this enzyme are theoretically possible, e.g. carbamazepine, dexamethasone, diclofenac, ibuprofen and naproxen.

**Adverse reactions.** Amaryl is generally well tolerated. Clinical experience has shown that adverse reactions serious enough to require discontinuation of therapy are uncommon, even during long-term therapy.

**Hypoglycaemia.** Hypoglycaemia is the greatest potential risk with all oral hypoglycaemics. Possible symptoms include headache, ravenous hunger, sweating, tinnitus, lassitude, sleepiness, disordered sleep, restlessness, aggressiveness, impaired concentration, impaired alertness and reactions, depression, confusion, speech disorders, aphasia, visual disorders, tremor, pareses, sensory disturbances, dizziness, faintness, loss of self-control, delirium, cerebral convulsions, somnolence and loss of consciousness up to and including death. Shallow respiration and bradycardia.

**Other signs of adrenergic counter-regulation may be present, including sweating, clammy skin, anxiety, tachycardia, hypertension, tachycardia, angina pectoris and cardiac arrhythmias.**

The clinical picture of a severe hypoglycaemic attack may resemble that of a myocardial infarction.

**Visual disturbances.** Visual disturbances nearly always subside when hypoglycaemia is corrected.

**Visual disturbances.** Especially at the start of treatment, there may be temporary visual impairment (e.g. changes in accommodation and/or depth vision) due to the change in blood glucose levels. The cause is a temporary alteration in the turgidity and hence the refractive index of the lens, this being dependent on blood glucose level.

**Gastrointestinal reactions.** Occasionally (0.1 to 1% of patients), gastrointestinal symptoms such as nausea, vomiting, sensations of fullness in the epigastrium, abdominal pain and diarrhoea may occur.

**Haematological reactions.** Anaemia, leucopenia, thrombocytopenia, leukocytopenia, purpura, agranulocytosis, pancytopenia due to aplastic anaemia, eosinophilia, haemolytic anaemia, aplastic anaemia, cytopenia and granulocytopenia have been reported with sulfonylureas.

**Dermatological reactions.** Allergic or pseudo-allergic skin reactions (e.g. pruritus, erythema, urticaria, erythematous and maculopapular and bullous skin eruptions or psoriasisiform drug eruption) may occur in patients treated with sulfonylureas. If skin reactions persist, the drug should be discontinued. Mild reactions in the form of urticaria may develop into serious and even life-threatening reactions with dyspnoea and hypotension, sometimes progressing to shock. In the event of urticaria, the doctor must be notified immediately. Porphyria cutanea tarda and pellagra-like changes and photosensitivity reactions have been reported with sulfonylureas. It should be noted that cross reactivity exists between sulfonylureas and sulphonamides.

**Heaptic reactions.** In isolated cases, increased liver enzymes (AST, ALT), abnormal liver function, cholestasis, cholestatic hepatitis, granulomatous hepatitis, bilirubinaemia and liver failure have been reported with sulfonylureas.

**Electrolyte disturbance.** In isolated cases, hyponatraemia has been reported in patients receiving glimepiride and other sulfonylureas, most often in patients who are on other medications or have medical conditions known to cause hyponatraemia or to increase release of antidiuretic hormone.

**Other.** Isolated cases of allergic vasculitis have been reported with sulfonylureas.

**Dosage and Administration.** In the management of type II diabetes mellitus, administration of an oral antidiabetic agent is not a substitute for appropriate dietary control.

In initiating treatment for noninsulin dependent diabetes mellitus (NIDDM), diet should be emphasised as the primary form of treatment. Caloric restriction and weight loss are essential in the obese diabetic patient. Proper dietary management alone may be effective in controlling the blood glucose and symptoms of hyperglycaemia. The importance of regular physical activity should also be stressed, and cardiovascular risk factors should be identified and corrective measures taken where possible.

If this treatment program fails to reduce symptoms and/or blood glucose, the use of an oral sulfonylurea should be considered. Use of Amaryl must be viewed by both the doctor and patient as a treatment in addition to diet, and not as a substitute for diet or as a convenient mechanism for avoiding dietary restraint. Furthermore, loss of blood glucose control on diet alone may be transient, thus requiring only short-term administration of Amaryl. During maintenance programs, Amaryl should be discontinued and insulin therapy initiated if satisfactory lowering of blood glucose is no longer achieved. Judgments should be based on regular clinical and laboratory evaluations.

The dosage of Amaryl must be the lowest which is sufficient to achieve the desired metabolic control. Dosage must be based on regular blood and urine glucose determinations, and must be carefully individualised to obtain optimum therapeutic effect. Periodic measurement of glycosylated haemoglobin is also recommended to monitor the patient's response to treatment. If appropriate glimepiride dosage regimens are not followed, hypoglycaemia may be precipitated.

Measures for dealing with errors in dosage such as forgetting to take a dose, skipping a meal or inability to take a dose at the prescribed time should be discussed with the patient at the time of initiating therapy. A missed dose must never be corrected by subsequently taking a larger dose.

Short-term administration of Amaryl may be sufficient during periods of transient loss of metabolic control in patients usually well controlled on diet.

Food does not alter the bioavailability or other pharmacokinetic parameters of glimepiride.

**Initial dose and dose titration.** The initial dose of Amaryl is one 1 mg tablet once daily. The tablet should be swallowed whole with adequate liquid (e.g. half a glass of water) immediately before breakfast. Patients who eat only a light breakfast should defer the first dose of the day until the first main meal of the day (e.g. lunch). It is very important that meals are not skipped after the tablet has been taken.

If good metabolic control is achieved within the first week of treatment (as determined by blood and urine glucose), continue the daily dose of one 1 mg tablet as maintenance therapy.

If metabolic control is unsatisfactory after one to two weeks of treatment, increase the daily dose in increments of 1 mg at one to two week intervals, until satisfactory metabolic control is achieved. Most patients will achieve optimum control at doses of 1 to 4 mg once daily. Only in exceptional cases will doses of more than glimepiride 4 mg/day give better results. Normally, a single daily dose will maintain good blood glucose control for 24 hours.

**Secondary dosage adjustment.** Amaryl requirements may fall as treatment proceeds because an improvement in diabetes control results in greater insulin sensitivity. To avoid hypoglycaemia, timely dose reduction or cessation of therapy should be considered.

Correction of Amaryl dosage must also be considered whenever the patient's weight or lifestyle changes or other factors arise which affect glycaemic control.

Secondary failures should be treated by discontinuing Amaryl and starting insulin.

**Changeover from other antidiabetic agents to Amaryl.** There is no exact dosage relationship between Amaryl and other oral antidiabetic agents. When transferring patients from another oral antidiabetic drug to Amaryl, it is recommended to begin with the usual starting dose of 1 mg once daily. This recommendation applies even in cases where the patient is being switched from the maximum dose of another antidiabetic agent.

Depending on the pharmacokinetic and pharmacodynamic characteristics of the previous medication, a drug free transition period may be necessary in order to avoid overlapping drug effects possibly resulting in hypoglycaemia.

**Renal impairment.** In patients with mild to moderate renal impairment, a starting dose of 1 mg once daily must not be exceeded. The dose may then be carefully titrated upwards if necessary based on fasting blood glucose levels according to the protocol mentioned above (i.e. in increments of 1 mg at intervals of one to two weeks).

No experience has been gained in the use of Amaryl in dialysis patients or patients with severe renal impairment. These patients should be changed over to insulin therapy to achieve optimum metabolic control.

**Heaptic Impairment.** No experience has been gained in the use of Amaryl in patients with severe hepatic impairment. These patients should be changed over to insulin therapy to achieve optimum metabolic control.

**Overdosage Symptoms.** Accidental or intentional overdose may cause severe and prolonged hypoglycaemia which may be life-threatening.

**Treatment.** In case of overdosage with glimepiride, a doctor must be notified immediately. At the first signs of hypoglycaemia, the patient must immediately take sugar, preferably glucose, unless a doctor has already started care.

Since hypoglycaemia and its clinical symptoms may recur after apparent clinical recovery (even after several days), close and continued medical supervision and possibly referral to a hospital are indicated. In particular, significant overdosage and severe reactions, e.g. with unconsciousness or other neurological dysfunctions, are emergency cases and require immediate care and hospitalisation. If hypoglycaemia coma is diagnosed or suspected intravenous infusion of a glucose 20% solution (adults: 40 to 100 mL) is indicated. Alternatively, intravenous, subcutaneous or intramuscular administration of glucagon (adults: 0.5 to 1 mg) may be considered. In infants, glucose must be dosed very carefully and close monitoring of blood glucose is required to minimise the risk of potentially severe hyperglycaemia. Other symptomatic therapy (e.g. anticonvulsants) should be administered as necessary.

After acute glucose replacement has been completed, it is usually necessary to give an intravenous glucose infusion in lower concentration so as to ensure that hypoglycaemia does not recur. The patient's blood glucose level should be carefully monitored for at least 24 hours.

In cases of acute intake of large amounts of glimepiride, detoxification (e.g. by gastric lavage and administration of medicinal charcoal) is indicated.

**Presentation.** Tablets, 1 mg (pink, oblong, scored, marked NMK and Hoechst logo on both sides), 2 mg (green, oblong, scored, marked NMM and Hoechst logo on both sides), 3 mg (yellow, oblong, scored, marked NMN and Hoechst logo on both sides), 4 mg (blue, oblong, scored, marked NMO and Hoechst logo on both sides); 10's (sample pack), 30's.

**Poisons Schedule S4.**

TGA approval/last amendment: 20/07/2001

## Avandia

Revised Entry this Edition

GlaxoSmithKline Australia

**Composition Active.** Rosiglitazone maleate.

**Inactive.** Sodium starch glycolate, hypromellose, microcrystalline cellulose, lactose and magnesium stearate. The film coat contains hypromellose, lactose, macrogol 3000, titanium dioxide, glycerol triacetate and the following colouring agents: 2 mg tablet: iron oxide red (CI77491); 4 mg tablet: purified talc, iron oxide yellow (CI77492) and iron oxide red (CI77491); 8 mg tablet: iron oxide red (CI77491).

**Description.** Chemical name: (*t*)-5-[4-[2-(methyl-2-pyridylamino) ethoxy]phenyl]-methyl]-2,4-thiazolidinedione, (Z)-2-butenoate (1:1). MW: 473.52 (357.44 free base). CAS: 0155141-29-0. Rosiglitazone maleate is a white to off-white solid with a melting range of 122 to 123°C. It is readily soluble in ethanol and a buffered aqueous solution with pH of 2.3; solubility decreases with increasing pH in the physiological range.

**Actions.** Oral antidiabetic agent.

**Pharmacology.** Avandia is a selective and potent agonist at the PPAR $\gamma$  (peroxisomal proliferator activated gamma) nuclear receptor and is a member of the thiazolidinedione class of antidiabetic agents. Avandia improves glycaemic control by improving insulin sensitivity at key sites of insulin resistance, namely adipose tissue, skeletal muscle and liver. Insulin resistance is known to play a major role in the pathophysiology of type 2 diabetes. Thus, Avandia improves metabolic control by lowering blood glucose, circulating insulin and free fatty acids.

The antihyperglycaemic activity of Avandia has been demonstrated in a number of rodent models of type 2 diabetes. In addition, Avandia preserved  $\beta$ -cell function as shown by increased pancreatic islet mass and insulin content and prevented the development of overt hyperglycaemia in rodent models of type 2 diabetes. Avandia has also been shown to significantly delay the onset of renal dysfunction and systolic hypertension. Avandia did not stimulate pancreatic insulin secretion or induce hypoglycaemia in rats and mice.

**Clinical trials.** Evidence to support the efficacy of Avandia in the treatment of type 2 diabetes was obtained in ten multicentre, double blind, placebo controlled studies and in one double blind comparator controlled study. These studies, conducted in a total of 3,462 patients, investigated the use of Avandia as monotherapy, and in combination with sulfonylureas (SUs) and metformin.

In clinical studies with Avandia given as monotherapy at doses of 4 to 8 mg/day, the glucose lowering effects are gradual in onset and are not associated with hypoglycaemia. Reductions in fasting plasma glucose are observed from one week of initiation of therapy, although the full therapeutic effect may take six to eight weeks to occur. While improvement in glycaemic control was associated with increases in weight, changes were highly variable. In 26 week clinical trials, the mean weight gain in patients treated with Avandia was 1.2 kg (range -11.6 to 12.7) (4 mg daily) and 3.5 kg (range -6.8 to 13.9) (8 mg daily) when administered as monotherapy, 0.7 kg (range -6.8 to 9.8) (4 mg daily) and 2.3 kg (range -5.4 to 13.1) (8 mg daily) when administered in combination with metformin and 1.8 kg (range -5 to 11.5) (4 mg daily) when administered in combination with sulfonylurea. In a 52 week glibenclamide controlled study, there was a mean weight gain of 1.75 kg (range -7.0 to 16.0) and 2.95 kg (range -11.0 to 22.0) for

## 6e Hypoglycaemic agents

patients treated with 4 and 8 mg of Avandia daily, respectively, versus 1.9 kg (range -11.5 to 12.2) in glibenclamide treated patients. Weight gain with thiazolidinediones can result from increases in subcutaneous adipose tissue and/or from fluid retention. Treatment should be re-evaluated in patients with excessive weight gain.

In type 2 diabetes, long term and sustained improvements in glycaemic control (FPG and HbA1c) have been demonstrated with Avandia given once or twice daily as monotherapy or in combination with other oral antidiabetic agents. In two studies, Avandia produced significantly greater reductions in FPG than glibenclamide after 52 weeks of treatment. Avandia treatment has been associated with clinically significant reductions in fasting and postprandial plasma glucose levels and in glycated haemoglobin. The improvement in glycaemic control was maintained throughout the duration of the studies (up to 18 months).

Consistent with the mechanism of action of Avandia, enhanced glycaemic control is accompanied by clinically significant decreases in serum insulin and C-peptide levels. There are also reductions in proinsulin and 32,33 split proinsulin, which are believed to correlate with cardiovascular risk factors. Significant decreases in free fatty acids are a key feature of Avandia treatment.

Patients with lipid abnormalities were not excluded from clinical trials of Avandia. As monotherapy, Avandia was associated with dose ordered increases in total cholesterol (TC), LDL-cholesterol and HDL-cholesterol and decreases in free fatty acids. The increase in LDL occurred during the first one to two months of therapy with Avandia, plateauing thereafter. In contrast, HDL continued to rise over time. As a result the TC/HDL ratio was unchanged after 12 months' treatment, with a subsequent reduction from baseline after longer term treatment. The pattern of LDL and HDL changes following therapy with Avandia in combination with metformin or sulfonylureas was generally similar in magnitude and time course to those seen with Avandia as monotherapy. The changes seen in triglycerides during therapy with Avandia were variable. See Table 1.

**Avandia**  
Table 1  
Summary of median lipid changes in a 52 week glibenclamide controlled monotherapy study (O20)

	Glibenclamide	Rosiglitazone 8 mg daily
Free fatty acids		
n	168	145
Baseline median (mmol/L)	0.92	0.93
% change	-9.7%	-24.7%
LDL		
n	160	133
Baseline median (mmol/L)	3.68	3.63
% change	-3.3%	+7.3%
HDL		
n	170	145
Baseline median (mmol/L)	1.18	1.19
% change	+8.0%	+17.4%
TC/HDL		
n	184	170
Baseline median (mmol/L)	4.90	4.76
Difference from baseline	-0.33	-0.08

**Monotherapy.** A total of 2,526 patients were treated with Avandia as monotherapy in six randomised, double blind, placebo/active controlled studies. These studies ranged in duration from 8 to 52 weeks and included patients with a range of severity of diabetes. Although a specific study of Avandia in diet and exercise treated patients has not been conducted, a total of 736 patients previously treated with diet and exercise alone received Avandia as monotherapy in the clinical trial program.

In a 26 week double blind, placebo controlled trial in type 2 diabetic patients with inadequate glycaemic control, all doses of Avandia resulted in significant improvement in glycaemic control relative to baseline and placebo (see Table 2).

When Avandia was dosed at 4 mg bd (twice daily), 70% of patients responded with a  $\geq 1.7$  mmol/L reduction from baseline in FPG compared to 58% treated with 8 mg od (once daily), 54% with 2 mg bd, 45% with 4 mg od and 19% with placebo.

When administered at the same total daily dose, Avandia was generally more effective when administered in divided doses twice daily compared to once daily doses. The effect on HbA1c, however, was not statistically significant between 4 mg once daily and 2 mg twice daily.

**Avandia**  
Table 2  
Improvement in metabolic control in a 26 week placebo controlled monotherapy study.

Study 024	Placebo	Avandia 4 mg od	Avandia 2 mg bd	Avandia 8 mg od	Avandia 4 mg bd
n	173	180	186	181	187
FPG (mmol/L)					
Baseline	12.5	12.7	12.5	12.7	12.7
Week 26	12.0	11.4	10.5	9.6	9.6
Difference from placebo (adjusted mean)	-	-1.7*	-2.4*	-2.7*	-3.4*
HbA1c (%)					
Baseline (mean)	8.93	8.91	8.87	8.94	9.04
Week 26 (mean)	9.72	8.93	8.74	8.62	8.73
Difference from placebo (adjusted mean)	-	-0.77*	-0.93*	-1.10*	-1.45*

\*p < 0.0001 compared to placebo.

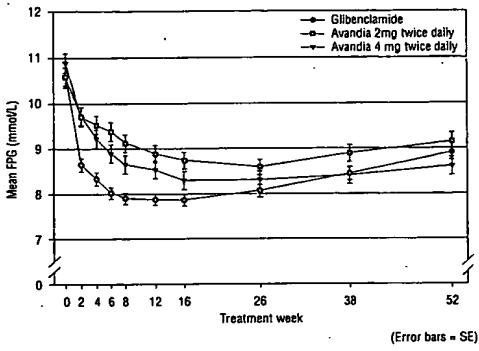
In a second 26 week double blind, placebo controlled study in diabetic patients with inadequate glycaemic control, a statistically significant improvement in HbA1c of 1.2 and 1.5% and in FPG of 3.2 and 4.2 mmol/L was observed with Avandia when administered at 2 mg od and 4 mg bd, respectively, compared with placebo.

When Avandia was dosed at 4 mg bd, 64% of patients responded with a 1.7 mmol/L reduction from baseline in FPG compared to 54% with 2 mg bd and 16% with placebo.

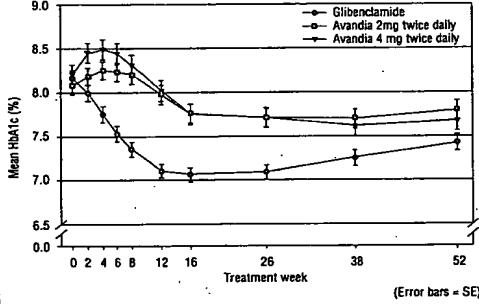
In a 52 week double blind, active controlled study which enrolled 587 type 2 diabetic patients, Avandia 2 mg bd and 4 mg bd was compared to glibenclamide. Patients in all three treatment groups displayed a statistically significant improvement in glycaemic control. According to the protocol defined definition of equivalence, Avandia 4 mg bd was as effective as glibenclamide in reducing HbA1c and resulted in significantly greater reductions in FPG than glibenclamide after 52 weeks of treatment. At the end of week 52, the reduction from baseline in FPG and HbA1c was 2.3 mmol/L and 0.53% with 4 mg bd Avandia; 1.4 mmol/L and 0.27% with 2 mg bd Avandia; 2.0 mmol/L and 0.72% with glibenclamide. The improvement in glycaemic control observed at week 26 with Avandia 4 mg bd was maintained throughout the second 26 week period of the study. In patients treated with Avandia, C-peptide, insulin and split products of insulin were significantly reduced, compared to an increase in the glibenclamide treated patients. (See Figures 1 and 2.)

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**Avandia**  
Figure 1  
Mean FPG over time in a 52 week glibenclamide controlled study



**Avandia**  
Figure 2  
Mean HbA1c over time in a 52 week glibenclamide controlled study



**Combination therapy with metformin and sulfonylureas.** Five well controlled double blind studies of 26 weeks duration assessed the efficacy of Avandia in combination with metformin or sulfonylureas (SUs). A total of 338 type 2 diabetic patients were treated concomitantly with Avandia and metformin and a total of 726 patients were treated concomitantly with Avandia and an SU. As a consequence of different but complementary mechanisms of action, combination therapy of Avandia with metformin or an SU resulted in additive improvements in glycaemic control in type 2 diabetic patients. The dose response relationship for efficacy was similar to that seen in monotherapy.

Improvements in metabolic control observed in two studies when Avandia was used in combination with a near maximal dose (2.5 g/day) metformin are displayed in Table 3.

**Avandia**  
Table 3  
Improvement in metabolic control in patients receiving Avandia plus metformin

	Avandia 4 mg od + metformin n = 116	Avandia 8 mg od + metformin n = 110	Avandia 4 mg bd + metformin n = 103
Change in FPG versus metformin alone	-2.2 mmol/L	-2.9 mmol/L	-3.1 mmol/L
Change in HbA1c versus metformin alone	-1.0%	-1.2%	-0.8%
% of patients with FPG reduction $\geq 1.7$ mmol/L	45%	60%	67%

Similarly, investigation of Avandia in three studies in combination with SU demonstrated that combination therapy with Avandia 2 mg twice daily resulted in significant decreases of up to 1.4% in HbA1c and of up to 3.1 mmol/L in FPG compared to SU alone.

Long-term studies on morbidity (including cardiovascular effects) and mortality outcomes are not yet available.

**Pharmacokinetics. Absorption.** Avandia is rapidly and completely absorbed after oral administration, with negligible first pass metabolism. Absolute bioavailability of Avandia following both a 4 and an 8 mg oral dose is approximately 99%. Plasma concentrations of Avandia peak at around one hour after dosing and are approximately dose proportional over the therapeutic dose range.

Administration of Avandia with food resulted in no change in overall exposure (AUC), although a small decrease in Cmax (approximately 20 to 28%) and a delay in Tmax (1.75 hours) were observed when compared to dosing in the fasted state. These small changes are not clinically significant and, therefore, it is not necessary to administer Avandia at any particular time in relation to meals. The absorption of Avandia is not affected by increases in gastric pH.

**Distribution.** The volume of distribution of Avandia is approximately 0.184 L/kg and total plasma clearance around 3 L/hour in healthy volunteers. Avandia is approximately 99.8% bound to plasma protein, primarily albumin. Concentration or age does not influence plasma protein binding of Avandia. There is no evidence for unex-

pected accumulation of rosiglitazone after once daily or twice daily dosing.

**Metabolism.** Metabolism of Avandia is extensive without a major compound being excreted unchanged. The major routes of metabolism are N-demethylation and hydroxylation, followed by conjugation with sulfate and glucuronic acid. The metabolites of Avandia are not considered to have any clinical relevance.

*In vitro* data demonstrate that Avandia is predominantly metabolised by cytochrome P450 (CYP) isoenzyme 2C8, with CYP2C9 contributing only as a minor pathway. In *in vitro* studies, rosiglitazone causes a moderate inhibition of CYP2C8 and minor inhibition of CYP2C9.

Significant inhibition of these enzymes is unlikely to occur at therapeutic doses. In addition, there is no significant *in vitro* inhibition of CYP1A2, 2A6, 2C19, 2D6, 2E1, 3A or 4A with Avandia, therefore there is a low probability of significant metabolism based interactions with drugs metabolised by these P450 enzymes (see Interactions).

**Excretion.** The terminal elimination half-life of Avandia is approximately three to four hours. The major route of excretion is the urine, with approximately two-thirds of the dose being eliminated by this route. Faecal elimination accounts for approximately 25% of the dose.

In the pooled population pharmacokinetic analysis, there were no marked differences in the pharmacokinetics of Avandia between males and females, or between elderly and non-elderly patients. In patients with moderate to severe (Child-Pugh B/C) hepatic impairment, unbound Cmax and AUC were two and threefold higher, respectively, in patients with hepatic impairment as a result of decreased protein binding and reduced clearance of Avandia (see Dosage and Administration).

There are no clinically significant differences in the pharmacokinetics of Avandia in patients with renal impairment or end stage renal disease on chronic dialysis. No dosage adjustment is required in these patients.

**Indications** Treatment of type 2 diabetes mellitus (non-insulin dependent diabetes mellitus).

It may be used as monotherapy in patients inadequately controlled by diet and exercise and in combination with sulfonylureas or metformin to improve glycaemic control in patients with type 2 diabetes mellitus.

**Contraindications** Hypersensitivity to rosiglitazone, male patients with any of the listed excipients.

**Precautions** Avandia is effective only in the presence of insulin resistance and should not be used in type 1 diabetes mellitus.

As a consequence of improving insulin sensitivity, Avandia treatment in premenopausal anovulatory patients with insulin resistance (patients with polycystic ovary syndrome) may result in resumption of ovulation. In these patients adequate contraception should be recommended to avoid the risk of pregnancy.

Premenopausal women have received Avandia during clinical studies. Although hormonal imbalance has been seen in premenopausal women (see Carcinogenicity, mutagenicity and impairment of fertility), no significant adverse experiences associated with menopausal disorders have been observed. If unexpected menstrual dysfunction occurs the benefits of continued therapy should be reviewed.

Patients with New York Heart Association (NYHA) class III or IV cardiac status were not studied during clinical trials. Rosiglitazone should not be used in these patients.

In patients with class I to II heart failure the benefits of therapy should be weighed against the risks before initiating treatment.

Since thiazolidinediones can cause fluid retention, which can exacerbate congestive heart failure, patients at risk for heart failure (particularly those on insulin) should be monitored for signs and symptoms of heart failure. Patients and/or their carers should be warned of the potential symptoms of worsening cardiac function if rosiglitazone should be discontinued if any deterioration is observed.

In controlled clinical trials of patients with type 2 diabetes, moderate oedema was reported in patients treated with Avandia, which may be dose related.

In clinical trials with Avandia, encompassing 2,492 patient years of exposure, there was no evidence of drug induced hepatotoxicity, elevations of ALT levels. In postmarketing experience with Avandia, there have been rare reports of hepatocellular dysfunction, primarily evidenced by elevated hepatic enzymes. Causality has not been established. However, it is recommended that patients treated with Avandia undergo periodic monitoring of liver enzymes. Liver enzymes should be checked prior to the initiation of therapy with Avandia in all patients. Therapy with Avandia should not be initiated in patients with increased baseline liver enzyme levels (ALT > 2.5 times the upper limit of normal).

In patients with normal baseline liver enzymes, following initiation of therapy with Avandia, it is recommended that liver enzymes should be monitored every two months for the first 12 months, and periodically thereafter. Patients with mildly elevated liver enzymes (ALT levels up to 2.5 x upper limit of normal) at baseline or during therapy with Avandia should be evaluated to determine the cause of the elevation. If any time ALT levels increase to  $> 3$  times the upper limit of normal in patients on therapy with Avandia, liver enzymes should be rechecked as soon as possible. If ALT levels remain elevated for  $> 3$  x the upper limit of normal, therapy with Avandia should be discontinued.

If any patient develops symptoms suggesting hepatic dysfunction, which may include unexplained nausea, vomiting, abdominal pain, fatigue, anorexia and/or dark urine, liver enzymes should be checked.

The decision whether to continue the patient on therapy with Avandia should be guided by clinical judgement pending laboratory evaluations. If jaundice is observed, drug therapy should be discontinued.

For patients with normal hepatic enzymes who are switched from troglitazone to Avandia, a one week washout is recommended before starting therapy with Avandia.

**Impaired hepatic function.** Owing to a difference in the pharmacokinetic profile (see Actions, Pharmacokinetics) and limited experience, Avandia is not recommended in patients with moderate to severe hepatic impairment (Child-Pugh B/C). Therapy with Avandia

## 6e Hypoglycaemic agents

should be initiated if the patient exhibits clinical evidence of active liver disease or increased transaminase levels (ALT > 2.5 x upper limit of normal) at the start of therapy.

**Genotoxicity, mutagenesis, impairment of fertility.** Two genotoxicity studies were conducted in Charles River CD-1 mice. At oral gavage doses of 0.1, 0.5, 1, 6 and 6 mg/kg/day in the diet and in water, no effects were seen at oral gavage doses of 0.05, 0.3 and 2 mg/kg/day. The highest oral gavage doses equivalent to approximately 10 to 20 times the maximum recommended human dose (under the curve) at the maximum recommended daily dose (16 mg/day). Rosiglitazone was not carcinogenic in the rat at doses > 1.6 mg/kg/day (approximately two times the maximum recommended human dose). There was a significant increase in incidence of adipose hyperplasia in rats at doses > 1.6 mg/kg/day (approximately two times the maximum recommended human dose). There was a significant increase in the incidence of benign tumours (lipomas) at doses > 0.3 mg/kg/day (approximately two times human AUC). These proliferative changes have been considered due to the persistent pharmacological action of rosiglitazone on adipose tissue.

Rosiglitazone was not mutagenic or clastogenic in the *in vitro* bacterial mutagenicity, the *in vitro* chromosome aberration test and the *in vivo* mouse micronucleus test and the *in vitro* Ames LUDS assay. There was a small (about twofold increase) inhibition in the *in vitro* mouse lymphoma assay at toxic concentrations of 160 to 200 microgram/mL.

No clinically relevant effects on healing performance or on fertility of male and female rats treated with rosiglitazone at exposures greater than those anticipated clinically (based on AUC). However, in the treatment of male rats with rosiglitazone, reduced weight gain was noted at exposures approximately twice the maximum recommended clinical exposure and this was associated with increased heart weight at exposures approximately ten times the maximum recommended clinical exposure (based on AUC).

Increased plasma levels of progesterone and oestradiol, reduced fertility of female rats but only at exposures to rosiglitazone greater than 20 times anticipated clinically (based on AUC). In monkeys, rosiglitazone diminished the surge in serum oestradiol with consequential reduction in the luteal phase surge, lower luteal phase progesterone and irregular menstrual cycles at exposures 2.7 times the maximum recommended clinical exposure (based on AUC).

In mice, increased heart weights were evident in mice (> 20 times the maximum recommended human dose, after two months' treatment) and rats (> 3 mg/kg/day after two months' treatment) after 12 months following treatment.

Cardiac angiography studies in patients with type 2 diabetes mellitus, receiving approximately 3, 2 and 0.3 times human recommended daily dose, respectively, demonstrated 'ballooning' of the hearts indicated ventricular dilation and reduced cardiac output. This phenomenon is considered to be due to increased workload as a result of plasma volume expansion.

Cardiac angiography studies in patients with type 2 diabetes mellitus, receiving 4 mg twice daily for 52 weeks ( $n = 86$ ) or 8 mg once daily for 26 weeks ( $n = 90$ ), have shown no significant alteration in cardiac structure or function. These studies were unable to detect a change in left ventricular mass of 10% or less, which is the threshold for detection.

In a study (Category B) rosiglitazone was not teratogenic in pregnant rabbits or rats during the period of organogenesis associated with respective exposures up to about 20 times those anticipated clinically (based on AUC). Following oral administration, rosiglitazone and/or its metabolites crossed the placenta and caused fetal death and retardation of fetal development in rats and rabbits. With no-effect doses of approximately four times the maximum recommended clinical exposure (based on AUC) in both species, no effect was observed on the embryo when rosiglitazone was given to pregnant rats or during implantation or early organogenesis.

From intermediate data and well controlled studies in pregnant women, Avandia should not be used during pregnancy unless the expected therapeutic benefit outweighs the potential risk to the fetus.

There is no information strongly suggests that abnormal blood glucose control in pregnancy are associated with a higher incidence of congenital anomalies, neonatal morbidity and mortality. The use of Avandia is not currently recommended for patients with type 2 diabetes mellitus who require it to maintain blood glucose levels as close to normal as possible.

**Pregnancy.** Following oral administration of radiolabelled rosiglitazone to lactating rats, the parent drug and/or its metabolites were found in the milk. It is not known whether rosiglitazone is secreted into human milk. Treatment of rats in pregnancy through lactation with rosiglitazone decreased postnatal survival, growth and weight gain in the offspring, with a maternal no-effect dose level associated with a plasma AUC of about three times the maximum recommended human value. Therefore, a decision should be made to discontinue breastfeeding or to discontinue the drug, taking into account the importance of the drug to the mother.

**Children.** There are no data available on the use of Avandia in children under 18 years of age, and therefore its use in this age group is not recommended.

**Effect on ability to drive or operate machinery.** Avandia does not cause drowsiness or sedation. It should not impair the ability to drive or operate machinery.

**Interactions.** No clinically relevant drug interactions have been reported with Avandia.

Concomitant administration with other oral antidiabetic agents including metformin, glibenclamide and acarbose did not result in any clinically significant pharmacokinetic or pharmacodynamic interactions.

Avandia has not been evaluated for use in combination with insulin. Avandia had no effects on the steady-state pharmacokinetics of metformin or glibenclamide nor did it affect the anticoagulant activity of warfarin.

Co-administration with ranitidine did not alter the pharmacokinetics of Avandia. The absorption of oral or intravenous doses of rosiglitazone, suggesting that the bioavailability of oral rosiglitazone is not altered by increases in

*in vitro* studies demonstrate that Avandia is predominantly metabolised by CYP2C8, with CYP2C9 contributing as only a minor pathway. Since there are no known inhibitors of CYP2C8 used clinically, concomitant drug therapy is not expected to inhibit rosiglitazone clearance. In addition, Avandia had no clinically relevant effect on the pharmacokinetics of St(-)-warfarin, a substrate for CYP2C9.

Rosiglitazone caused a moderate inhibition of CYP2C8 and a minor inhibition of CYP2C9 *in vitro*. Significant inhibition of these enzymes is unlikely to occur at therapeutic doses (see Actions, Pharmacokinetics). Since there are only a few known substrates for CYP2C8 (paclitaxel, cerivastatin), the potential for an interaction involving this enzyme is even more unlikely.

No clinically relevant effects on nifedipine or oral contraceptives (components ethynodiol-drostanol and norethindrone) were observed after coadministration with Avandia confirming a low probability of clinically relevant interactions with drugs metabolised by CYP3A4. Moderate ingestion of alcohol with Avandia has no effect on glycemic control.

**Adverse Reactions.** In clinical trials, adverse experiences with Avandia were generally not dose related, and were mostly mild and transient in nature. In placebo controlled studies, Avandia was well tolerated when used as monotherapy or in combination with SUs (sulfonylureas) and metformin. The need for discontinuation of therapy due to adverse experience occurred in 7.5% of patients treated with Avandia compared with 8.2% of placebo patients (in placebo controlled studies). (See Table 4.)

Table 4  
Most common reported adverse experiences (> 5% in any treatment group) in double blind monotherapy studies

Preferred term	Avandia monotherapy n = 2,526 n (%)	Placebo n = 601 n (%)	Metformin n = 225 n (%)	Sulfonylureas* n = 626 n (%)
Patients with adverse events	1742 (59.0%)	374 (62.2%)	172 (76.4%)	438 (70.0%)
Upper respiratory tract infection	251 (9.9%)	52 (8.7%)	20 (8.9%)	46 (7.3%)
Injury	192 (7.6%)	26 (4.3%)	17 (7.5%)	38 (6.1%)
Headache	148 (5.9%)	30 (5.0%)	20 (8.4%)	34 (5.4%)
Back pain	102 (4.0%)	23 (3.8%)	9 (3.9%)	51 (8.1%)
Hyperglycaemia	99 (3.9%)	31 (5.2%)	10 (4.4%)	12 (1.9%)
Fatigue	92 (3.6%)	30 (5.0%)	9 (4.0%)	16 (3.0%)
Sinusitis	82 (3.2%)	27 (4.5%)	12 (5.3%)	19 (3.0%)
Diarrhoea	59 (2.3%)	20 (3.3%)	35 (15.6%)	37 (5.9%)
Hypoglycaemia	16 (0.6%)	1 (0.2%)	3 (1.3%)	-

\* Includes patients receiving glibenclamide ( $n = 514$ ), glizide ( $n = 91$ ) or glipizide ( $n = 21$ ).

In a small number of patients treated with Avandia adverse experiences of oedema, hypercholesterolaemia and anaemia (decreased haemoglobin) were reported in double blind trials.

Oedema was reported in 4.8% of patients taking Avandia, compared to 1.3% on placebo, 1.0% on sulfonylureas and 2.2% on metformin. Treatment was required for 1.2% of patients with an adverse event of oedema. These adverse experiences uncommonly led to withdrawal. Hypercholesterolaemia was reported in 3.4% of patients. The elevated total cholesterol levels were associated with an increase in both LDLc ( $n = 2,048$ ) and HDLc ( $n = 2,177$ ) and the ratio of total cholesterol:HDLc was unchanged or decreased in long-term studies ( $n = 866$  after 12 months' therapy). Overall, these experiences were generally mild to moderate and usually did not require discontinuation of treatment.

Across all controlled clinical studies, decreases in haemoglobin and haematocrit (mean decreases in individual studies  $\leq 1.0$  g/dL and  $\leq 3.3\%$ , respectively) were observed for both Avandia alone and in combination with metformin or sulfonylurea. The changes occurred primarily during the first four to eight weeks of therapy and remained relatively constant thereafter. Anaemia (decreased haemoglobin) was reported at an incidence of 1.9% in double blind studies with Avandia. The incidence of anaemia was higher when Avandia was used in combination with metformin (7.1%). Lower pretreatment haemoglobin/haematocrit levels in patients enrolled in the metformin combination clinical trials may have contributed to the higher reporting rate of anaemia in these studies.

White blood cell counts also decreased slightly in patients treated with Avandia. The observed changes may be related to the increased plasma volume observed with treatment with Avandia and have not been associated with any significant haematological clinical effects. An increase in weight was reported as an adverse experience by 0.9% of patients on Avandia.

In a large clinical program (4,327 patients treated with Avandia) the incidence of elevations of ALT greater than three times the upper limit of normal was equal to placebo (0.2%) and less than that of the active comparators (0.5% metformin/SUs). The incidence of reports of all adverse experiences relating to liver and biliary systems also was low and equal to placebo (0.7%).

Dose related weight gain was seen with Avandia alone and in combination with other hypoglycaemic agents. The mechanism of weight gain is unclear but probably involves a combination of fluid retention and fat accumulation (see Actions, Clinical trials).

In keeping with its mechanism of action, Avandia given as monotherapy is not associated with hypoglycaemia. In clinical trials, Avandia did not potentiate the hypoglycaemic effects of sulfonylureas. However, patients receiving rosiglitazone in combination with insulin or oral hypoglycaemic agents may be at risk for hypoglycaemia and a reduction in the dose of the concomitant agent may be necessary.

Postmarketing reports of congestive heart failure and pulmonary oedema have been received rarely.

Postmarketing reports of hepatic dysfunction, primarily evidenced by elevated hepatic enzymes, have been received rarely. A causal relationship to rosiglitazone has not been established.

**Dosage and Administration.** Avandia therapy can be initiated at 4 mg/day. This dose can be increased to 8 mg/day after six to eight weeks if greater glycaemic control is required. Avandia may be given once or twice a day (see Actions, Clinical trials).

Avandia may be taken with or without food.

No dose adjustment is required in the elderly. No dose adjustment is required in patients with varying degrees of renal insufficiency, including patients with end stage renal disease on chronic haemodialysis.

No dosage adjustment is required in patients with mild hepatic impairment (Child-Pugh A). Owing to a difference in pharmacokinetics and limited experience, Avandia is not recommended in patients with moderate to severe hepatic impairment (Child-Pugh B/C). Therapy with Avandia should not be initiated if the patient exhibits clinical evidence of active liver disease or increased transaminase levels (ALT > 2.5 x upper limit of normal) at the start of treatment (see Precautions).

**Overdosage.** Limited data are available with regard to overdosage in humans. In clinical studies in volunteers Avandia has been administered at single oral doses of up to 20 mg and was well tolerated. In the event of an overdose, it is recommended that appropriate supportive treatment should be initiated as dictated by the patient's clinical status. Avandia is highly protein bound and is not cleared by haemodialysis.

**Presentation.** Tiltab tablets, 2 mg (film coated, pentagonal, pink); 14's, 28's, 56's (2 mg currently not available in Australia); 4.0 mg (film coated, pentagonal, orange); 7's, 28's, 56's (only 28's currently available in Australia); 8.0 mg (film coated, pentagonal, red-brown); 28's.

**Storage.** Store below 30°C. Shelf-life at this temperature is 2 years.

**Poisons Schedule S4.**

**TGA approval/last amendment:** 30/05/2002

### Daonil, Semi-Daonil

Revised Entry this Edition

Aventis Pharma Pty Limited

Composition Glibenclamide.

**Description.** Glibenclamide belongs to the sulfonylurea group of oral antidiabetics. Earlier members of this group are carbutamide, tolbutamide, acetohexamide and chlorpropamide.

Chemical name: 1-(4-(2-(5-chloro-2-methoxybenzamido)ethyl)benzenesulfonyl)-3-cyclohexylurea. Molecular formula:  $C_{23}H_{25}ClN_3O_5S$ . MW: 494. It is a white, odourless, crystalline powder, practically insoluble in water and in ether, slightly soluble in alcohol and sparingly soluble in chloroform.

**Actions.** Oral hypoglycaemic.

**Pharmacology.** Daonil appears to lower the blood glucose acutely in healthy individuals and type II diabetic patients by stimulating the release of insulin from the pancreas, an effect dependent upon the functioning  $\beta$  cells. It acts in concert with glucose (improved sensitivity of  $\beta$  cells to physiological glucose stimulus) and leads to insulin secretion in the rhythm of meals. Other mechanisms of the hypoglycaemic action associated with short-term therapy appear to include reduction of basal hepatic glucose production and enhancement of peripheral insulin action at postreceptor (probably intracellular) sites.

With chronic administration of Daonil and Semi-Daonil in type II diabetic patients, the improvement in glucose tolerance persists, despite a gradual decline in glucose or meal stimulated secretion of insulin towards pretreatment levels. Extrapancreatic effects appear to substantially contribute to the hypoglycaemic action of the drug during long-term administration. The effects appear to include enhanced peripheral sensitivity to insulin and reduction of basal hepatic glucose production. There is evidence that glibenclamide enhances the peripheral action of insulin at postreceptor (probably intracellular) sites and increases the insulin binding and/or the number of insulin receptors.

Glibenclamide also exerts a direct inhibitory effect on glucagon producing  $\alpha$  cells of the pancreas and increases the release of somatostatin. However, these two pancreatic extra- $\beta$  cell actions may play only a minor clinical role.

In addition to its blood glucose lowering effect, glibenclamide has a mild diuretic action and increases the free water clearance.

**Pharmacokinetics.** Absorption, metabolism and excretion. Glibenclamide is nearly completely absorbed ( $84 \pm 9\%$ ) after oral administration and is extensively bound (99%) to serum proteins. The peak serum concentration is reached in two to six hours after taking a Daonil 5 mg tablet and falls within 24 hours to less than 5% of the peak value. The area under the serum concentration-time curve (AUC) increases in proportion to increasing doses. Food apparently does not affect the rate or extent of absorption of glibenclamide.

Multiple dose studies with glibenclamide in diabetic patients demonstrate drug level concentration-time curves similar to single dose studies, indicating no buildup of drug in tissue depots. In nonfasting diabetic patients, the hypoglycaemic action of a single morning dose of glibenclamide persists for 24 hours.

Serum concentrations of glibenclamide appear to decline in a biphasic manner. The elimination half-life of glibenclamide after intravenous dosage is approximately two hours, and two to five hours after oral administration. Some reports indicate a longer half-life of eight to ten hours in diabetic patients.

Glibenclamide appears to be completely metabolised, probably in the liver. The drug is metabolised at the cyclohexyl ring principally to the 4-trans-hydroxy derivative. A second metabolite, the 3-cis-hydroxy derivative, also occurs. These metabolites contribute no significant hypoglycaemic action since they are only weakly active (0.25% and 2.5%, respectively, as glibenclamide) in rabbits.

Glibenclamide is excreted as metabolites in the bile and urine, approximately 50% by each route. In patients with renal insufficiency, depending on the degree of the renal excretion disorder, there is increased elimination of the metabolites via the bile. This dual excretory pathway is qualitatively different from that of other sulfonylureas, which are excreted primarily in the urine.

**Indications.** As an adjunct to diet to lower the blood glucose in patients with noninsulin dependent diabetes mellitus (type II) whose hyperglycaemia cannot be controlled by diet alone. Because of its broad and predictable action, Daonil and Semi-Daonil are often

## 6e Hypoglycaemic agents

suitable for the management of patients who have failed to respond to other oral antidiabetics.

In initiating treatment for noninsulin dependent diabetes, diet should be emphasised as the primary form of treatment. Caloric restriction and weight loss are essential in the obese diabetic patient. Proper dietary management alone may be effective in controlling the blood glucose and symptoms of hyperglycaemia. The importance of regular physical activity should also be stressed, and cardiovascular risk factors should be identified and corrective measures taken where possible. If this treatment program fails to reduce symptoms and/or blood glucose, the use of an oral sulfonylurea should be considered. Use of Daonil and Semi-Daonil must be viewed by both the doctor and patient as a treatment in addition to diet, and not as a substitute for diet or as a convenient mechanism for avoiding dietary restraint.

**Contraindications** Known hypersensitivity or allergy to glibenclamide. Insulin dependent diabetes (type I or juvenile onset diabetes) or diabetes complicated by ketosis. Serious metabolic decompensation with acidosis, in particular precoma and coma. Severe impairment of renal function. Severe hepatic dysfunction. Pregnancy (see *Precautions, Use in pregnancy*). Lactation (see *Precautions, Use in lactation*).

**Warnings** The treatment of diabetes requires regular checks. Until optimal control is achieved, or when changing from one product to another, or when tablets are not taken regularly, the patient's alertness and capacity to react may be impaired to such an extent that they may not be fit to drive or to operate machinery.

When situations of unusual stress arise (e.g. emergency or elective surgery, febrile infections), a temporary change to insulin may become necessary.

It should be borne in mind that there is a possibility of cross sensitivity to sulfonylureas and their derivatives.

Alcohol (see *Interactions*).

**Use in children.** The safety and efficacy of glibenclamide in children have not been established. Glibenclamide is not recommended for use in this age group.

**Precautions Hypoglycaemic reactions.** Severe hypoglycaemia, which may be prolonged and is potentially lethal, can be induced by all sulfonylureas.

Debilitated, malnourished or elderly patients and patients with mild disease or impaired hepatic or renal function should be carefully monitored and the dosage of glibenclamide should be carefully adjusted in these patients, since they may be predisposed to developing hypoglycaemia. Renal or hepatic insufficiency may cause increased serum concentrations of glibenclamide and hepatic insufficiency may also diminish glycogenetic capacity, both of which increase the risk of severe hypoglycaemic reactions.

Alcohol ingestion (see *Interactions*), severe or prolonged exercise, deficient caloric intake, use of more than one antidiabetic agent, severe endocrine disorders and adrenal or pituitary insufficiency may also predispose patients to the development of hypoglycaemia.

Patients receiving glibenclamide should be monitored with regular clinical and laboratory evaluations, including blood and urine glucose determinations, to determine the minimum effective dosage and to detect primary failure (inadequate lowering of blood glucose concentration at the maximum recommended dosage) or secondary failure (loss of control of blood glucose concentration following an initial period of effectiveness) to the drug. Glycosylated haemoglobin measurements may also be useful for monitoring the patient's response to glibenclamide therapy. During the withdrawal period in patients in whom glibenclamide is replacing insulin, patients should be instructed to test their urine for glucose and ketones at least three times daily, and to report the results to their doctor. When feasible, patient or laboratory monitoring of blood glucose concentration is preferable. Care should be taken to avoid ketosis, acidosis and coma during the withdrawal period in patients being switched from insulin to glibenclamide. If adequate lowering of blood glucose concentration is no longer achieved during maintenance therapy with glibenclamide, the drug should be discontinued.

Patients and responsible family members should be made aware of the signs and symptoms of hypoglycaemia (severe thirst, dry mouth, frequent micturition, dry skin) and hypoglycaemia (intense hunger, sweating, tremor, restlessness, irritability, depression, headaches, disturbed sleep or transient neurological disorders) and the prompt action to the occurrences.

In the presence of a genetic defect in metabolism, the elimination half-life may be prolonged.

Because of their broad and predictable hypoglycaemic effect, Daonil and Semi-Daonil should be taken immediately before breakfast. Patients who eat only a light breakfast should defer the first dose of the day until lunchtime.

Some improvement in glucose tolerance may take place after a few weeks' treatment with Daonil or Semi-Daonil. The clinical status should be checked within the first four to eight weeks and at regular intervals thereafter, so as to ascertain whether it is possible to cut down the dose.

**Use in pregnancy.** (Category C) It is important to achieve strict normoglycaemia during pregnancy. Oral hypoglycaemic agents should be replaced by insulin. The sulfonylureas may enter the fetal circulation and cause neonatal hypoglycaemia. In animal studies, embryotoxicity and/or birth defects have been demonstrated.

**Use in lactation.** It is not known whether glibenclamide is excreted in milk or whether it has a harmful effect on the newborn infant. Therefore it is not recommended for breastfeeding mothers unless the expected benefits outweigh any potential risks.

**Interactions** Other drugs given at the same time as sulfonylureas may cause undesirable depression or elevation of the blood sugar level.

Drugs that may potentiate the hypoglycaemic action of Daonil and Semi-Daonil include alcohol, angiotensin converting enzyme (ACE) inhibitors, aminosalicylic acid, anabolic steroids,  $\beta$ -receptor blockers, bezafibrate, biguanides, chloramphenicol, clofibrate, clonidine, combined trimethoprim/sulfamethoxazole (co-trimoxazole), coumarin derivatives, disopyramide, fenfluramine, fluoxetine, gemfibrozil,

guanethidine, heparin, MAOIs, miconazole, oxpentifyline (parenteral, in high doses), phenylbutazone, phényramidol, phosphamides, probenecid, quinolone antibiotics, ranitidine, reserpine, salicylates, sulfinpyrazone, tirofiban, tetracycline compounds and certain long acting sulfonamides. Highly protein bound drugs may also potentiate the hypoglycaemic action of Daonil and Semi-Daonil due to glibenclamide displacement from plasma proteins, including oral anticoagulants, hydantoin, salicylates and other NSAIDs.

Drugs that may cause an attenuation of the hypoglycaemic action of Daonil and Semi-Daonil include alcohol, acebutolol, calcium channel blockers, cimetidine, clonidine, diazoxide, corticosteroids, glucagon, isoniazid, nicotinic acid (high dosage), oestrogens, progestogens, phenothiazine derivatives, phenytoin, ranitidine, rifampicin, ritodrine, saluretics, sympathomimetic agents, thyroid hormones and large doses of laxatives.

Concomitant treatment with  $\beta$ -receptor blockers or clonidine may mask the warning symptoms of a hypoglycaemic attack. In rare instances, potentiation or attenuation of the blood sugar lowering effect of Daonil and Semi-Daonil has been observed during concomitant therapy with H<sub>2</sub>-receptor antagonists.

In very rare cases, an intolerance to alcohol may occur. Excessive alcohol ingestion by people who drink occasionally may attenuate the hypoglycaemic effect of glibenclamide or dangerously potentiate it by delaying its metabolic inactivation. Disulfiram-like reactions have occurred very rarely following the concomitant use of alcohol and glibenclamide.

Food does not alter bioavailability or other pharmacokinetic parameters of glibenclamide.

**Adverse Reactions** Clinical experience in the use of Daonil and Semi-Daonil has shown that side effects serious enough to compel discontinuation of therapy are uncommon, even during long-term therapy. However, if adverse effects persist, the drug should be discontinued.

**Hypoglycaemia.** May be not only severe, but also prolonged and fatal (see *Precautions and Overdosage*).

**Gastrointestinal reactions.** Adverse gastrointestinal effects, e.g. nausea, vomiting, epigastric fullness or sensation of pressure, anorexia, heartburn, dyspepsia and diarrhoea, are the most common adverse reactions to glibenclamide, occurring in about 1 to 2% of patients. Glibenclamide induced adverse gastrointestinal effects appear to be dose related and may subside following a reduction in dosage. Pancreatitis has been reported rarely.

**Dermatological reactions.** Allergic skin reactions, e.g. pruritus, erythema, urticaria, and erythema multiforme, maculopapular and bullous skin eruptions or psoriasis-like drug eruption, occur in 1.5% of treated patients. These may be transient and may disappear despite continued use of glibenclamide; if skin reactions persist, the drug should be discontinued. Porphyria cutanea tarda, pellagra-like changes and photosensitivity reactions have been reported with sulfonylureas.

**Haematological reactions.** Anaemia, leucopenia, thrombocytopenia, thrombocytopenic purpura, agranulocytosis, pancytopenia, eosinophilia, haemolytic anaemia, aplastic anaemia, bone marrow aplasia, eosinophilia and coagulation disorders have been reported with sulfonylureas.

**Hepatic reactions.** Increased liver enzymes (AST, ALT), abnormal liver function, cholestasis, cholestatic hepatitis, granulomatous hepatitis and bilirubinaemia have been reported with sulfonylureas.

**Miscellaneous.** Although a causal relationship has not been established, the following adverse effects have been reported in patients receiving glibenclamide: paraesthesia, blindness, deafness, diplopia, visual disturbances, tremor, convulsions (other than withdrawal), encephalopathy, confusion, acute psychosis, abnormal renal function, acute renal failure, ocular disturbances (accommodation changes, crystalline lens changes), lactic acidosis, alopecia/hypotrichosis, hypotension, syndrome of inappropriate secretion of antidiuretic hormone (SIADH), arthralgia, arthritis, cerebrovascular disorders, headache, facial oedema, angioedema, hypersensitivity vasculitis and increased sweating.

**Dosage and Administration** Dosage of glibenclamide must be based on blood and urine glucose determinations and must be carefully individualised to obtain optimum therapeutic effect. If appropriate glibenclamide dosage regimens are not followed, hypoglycaemia may be precipitated.

In newly treated diabetic patients, stabilisation should be commenced with one Semi-Daonil tablet daily, taken immediately before breakfast. Patients who eat only a light breakfast should defer the first dose of the day until lunchtime.

After three to five days the blood sugar and urine sugar should be checked. If good control has been achieved, the daily dose of one Semi-Daonil tablet is continued as maintenance therapy.

If control is unsatisfactory, elevation of the daily dose in steps of 2.5 mg is necessary, at intervals of seven days, up to a maximum of 15 mg or, in exceptional cases, four tablets (20 mg) daily.

Daily allotments of up to 10 mg can be taken as a single dose before breakfast; daily dosage in excess of 10 mg should be taken before the evening meal.

In the management of type II diabetes mellitus, oral hypoglycaemic administration is not a substitute for appropriate dietary control.

When transferring patients from other oral antidiabetic drugs, it is recommended to begin with the usual starting dose (2.5 to 5 mg/day). Depending on the pharmacokinetic and pharmacodynamic characteristics of the previous medication, a drug free transition period may be necessary in order to avoid overlapping drug effects, possibly resulting in hypoglycaemia.

In general, patients who were previously maintained on insulin dosages up to 40 IU daily may be transferred directly to glibenclamide and administration of insulin may be abruptly discontinued; the initial glibenclamide dosage is 2.5 to 5 mg daily in patients whose insulin dosage was less than 20 IU daily and 5 mg daily in patients whose insulin dosage was 20 to 40 IU daily. In patients requiring insulin dosages greater than 40 IU daily, an initial glibenclamide dosage of 5 mg daily should be started and the insulin dosage reduced by 50%. Subsequently, insulin is withdrawn gradually and dosage of glibenclamide

is increased in increments of 1.25 to 2.5 mg daily every 1 to 2 days, according to the patient's tolerance and therapeutic response. During the period of insulin withdrawal, patients should test themselves at least three times daily for glucose and acetone, and should be instructed to report the results to their doctor so that appropriate adjustments in therapy may be made. If necessary, when baseline patient or laboratory monitoring of blood glucose concentration is preferable. The presence of persistent ketonuria with glycosuria and/or inadequate lowering or persistent elevation of blood glucose concentration indicates that the patient requires insulin therapy.

If adequate control is no longer possible with diet and Daonil or Semi-Daonil (maximum 20 mg daily), good results may be obtained by combined administration of Daonil and a biguanide derivative.

**Overdosage Pathogenesis.** Acute glibenclamide toxicity may result from excessive dosage, and numerous conditions may predispose patients to the development of glibenclamide induced hypoglycaemia (see *Contraindications, Warnings and Precautions*). Acute fatal and intentional overdose of glibenclamide may cause severe and prolonged hypoglycaemia. Fatal hypoglycaemia has occurred following ingestion of as little as 2.5 to 5 mg of the drug.

**Symptoms.** Acute glibenclamide overdose is manifested principally as hypoglycaemia, which may be severe and has occasionally been fatal. Severe hypoglycaemia may result in loss of consciousness and seizures, with resultant neurological sequelae.

**Treatment.** In case of overdosage with glibenclamide, a doctor should be called immediately. At the first signs of hypoglycaemia, the patient must immediately take sugar, preferably glucose, unless the doctor has already started care.

Since hypoglycaemia and its clinical symptoms may be subtle, apparent clinical recovery (even after several days) does not indicate continued medical supervision and possibly referral to a hospital is indicated. In particular, significant overdosage and severe reactions, e.g. with unconsciousness or other neurological dysfunction, emergency cases and require immediate care and hospitalisation.

If hypoglycaemic coma is diagnosed or suspected, administer Daonil (adults: 0.5 to 1 mg) intravenously, subcutaneously or orally, or an intravenous infusion of a 20% glucose solution (adults: 40 to 100 mL), until the patient recovers consciousness. In infants, glucose must be dosed very carefully, accompanied by close monitoring of blood glucose, taking into account the risk of potential severe hyperglycaemia. Other symptomatic therapy (e.g. anti-emetics) should be administered as necessary.

In cases of acute intake of large amounts of glibenclamide, e.g. by gastric lavage or medicinal charcoal as an antidote, as indicated.

**Presentation** Daonil. Tablets, 5 mg (white, elongated, scored sides, marked LD1 with Hoechst logo); 100's.

Semi-Daonil. Tablets, 2.5 mg (white, scored, marked with Hoechst logo); 100's.

**See Product Identification Guide.** Daonil 5 mg (grid 317), Daonil 2.5 mg (grid 267, 318).

**Poisons Schedule S4.**

**TGA approval/last amendment:** 17/12/1999

### Diabex

**Revised Entry this Edition**

Alphapharm Pty Ltd

Life-threatening lactic acidosis can occur due to accumulation of metformin. Risk factors include renal impairment. Other risk factors include old age associated with reduced renal function; and high doses of metformin above 2 g/day.

**Composition** Active: Metformin hydrochloride.

Inactive: Povidone, magnesium stearate, hypromellose.

1,000 mg tablets also contain Macrogol 400 and Macrogol 4000. Gluten free.

**Description** Chemical name: 1,1-dimethylbiguanide hydrochloride. Molecular formula: C<sub>4</sub>H<sub>10</sub>N<sub>4</sub>·HCl. MW: 165.6. CAS: 1-137-0.

Metformin hydrochloride is a white crystalline powder which is tasteless or almost odourless and hygroscopic. It is freely soluble in water, slightly soluble in ethanol (96%), and practically insoluble in chloroform and in ether.

**Actions** Oral biguanide antihyperglycaemic agent.

**Pharmacology.** Metformin causes an increased peripheral uptake of glucose by increasing the biological efficiency of available insulin or endogenous insulin.

The mode of action of metformin may be linked to an increase in insulin sensitivity. It does not stimulate insulin release but does not require the presence of insulin to exert its antihyperglycaemic effect.

Possible mechanisms of action include inhibition of gluconeogenesis in the liver, delay in glucose absorption from the gastrointestinal tract and an increase in peripheral uptake of glucose.

Metformin has an antiketogenic activity which is comparable though somewhat inferior, to insulin itself. Metformin lowers both basal and postprandial blood glucose in diabetic patients but does not cause hypoglycaemia in either diabetics or normal individuals.

**Clinical trials.** The prospective randomised (UKPDS) study established the long-term benefit of intensive blood glucose control in type 2 diabetes.

Analysis of the results for overweight patients treated with metformin after failure of diet alone showed the following: a significant reduction of the absolute risk of any diabetes related complication in the metformin group (29.8 events/1,000 patient years) versus diet alone (43.3 events/1,000 patient years), p = 0.0023, and versus the combined sulfonylurea and insulin monotherapy groups (44.6 events/1,000 patient years), p = 0.0034;

a significant reduction of the absolute risk of diabetes related mortality in the metformin 7.5 events/1,000 patient years, diet alone 12.7 events/1,000 patient years, p = 0.017;

a significant reduction of the absolute risk of overall mortality in metformin 13.5 events/1,000 patient years versus diet alone 20.8 events/1,000 patient years (p = 0.011), and versus the combined

## 6e Hypoglycaemic agents

risks, including overall alkalisation with sodium bicarbonate. Because metformin hydrochloride is dialysable (with a clearance of up to 170 mL/minute under good haemodynamic conditions), prompt haemodialysis is recommended to correct the acidosis and remove the accumulated metformin.

**Presentation** Tablets, 500 mg (white, scored, capsule shaped, film coated, marked DIABEX); 100's; 850 mg (white, film coated); 60's; 1,000 mg (white, scored, oval shaped, film coated, marked 1000); 60's.

See Product Identification Guide. Diabex 500 mg (grid 153); Diabex 1,000 mg (grid 853).

**Poisons Schedule S4.**

TGA approval/last amendment: 27/08/2002

### Diavormin

Alphapharm Pty Ltd

**Composition** Active. Metformin hydrochloride.

Inactive. Povidone, magnesium stearate, Dri Klear-010 (includes hypromellose, hydroxypropylcellulose, polyethylene glycol); gluten free.

**Presentation** Tablets, 500 mg (white, oblong, clear coated, scored, marked MF1): 100's; 850 mg (white, round, clear coated, marked MF 2, G on reverse); 60's.

See Product Identification Guide. Diavormin 500 mg (grid 349); Diavormin 850 mg (grid 350, 218).

For further information see other brands/ presentations of metformin hydrochloride or eMIMS.

### Diamicron

Servier Laboratories (Aust.) Pty Ltd

**Composition** Active. Gliclazide.

Inactive. Lactose, maize starch, purified talc, magnesium stearate, pregelatinised maize starch.

**Description** Chemical name: 1-(3-azabicyclo[3.3.0]oct-3-yl)-3-p-tolylsulfonylurea. Molecular formula:  $C_{15}H_{21}N_3O_3S$ . CAS: 21187-98-4. Melting point: approximately 168°C. CAS: 21187-98-4. Gliclazide is a white almost white powder, practically insoluble in water, freely soluble in dichloromethane, sparingly soluble in acetone and slightly soluble in ethanol 96%.

**Actions Pharmacology** Mode of action. Gliclazide stimulates insulin secretion from functional pancreatic  $\beta$ -cells and increases the sensitivity of the  $\beta$ -cells to a glucose stimulus (some residual  $\beta$ -cell function is therefore necessary). Gliclazide restores the diminished first-phase of insulin secretion noted in noninsulin dependent diabetes mellitus.

Any long-term hypoglycaemic activity of gliclazide can be attributed to an ability to maintain its effect on insulin secretion. Extrapancreatic effects may also be involved in the long-term efficacy of gliclazide. Extrapancreatic effects demonstrated for gliclazide include improvement in insulin mediated glucose utilisation and potentiation of post-receptor insulin sensitive pathways.

At normal therapeutic doses in humans, gliclazide reduces platelet adhesiveness and aggregation.

**Pharmacokinetics. Absorption**. Gliclazide is absorbed in the gastrointestinal tract reaching peak serum concentrations within four to six hours.

Single dose studies have demonstrated that maximal falls in blood glucose levels (23% of an 80 mg dose; 30% of a 160 mg dose) occur approximately five hours after drug administration; nine hours after a dose of 160 mg, a reduction of 20% was still in evidence.

The half-life of gliclazide is approximately 12 hours.

**Distribution**. Gliclazide is distributed to the extracellular fluid. In animals, high concentrations of the drug were found in the liver, kidneys, skin, lungs, skeletal muscle, intestinal and cardiac tissue. Penetration of gliclazide into the central nervous system was negligible. Gliclazide crosses the placental barrier and penetrates the fetus. The apparent volume of distribution of gliclazide (20 to 40% expressed as a percentage bodyweight) is low and probably reflects the high degree of protein binding (94.2% at a plasma concentration of approximately 8 microgram/mL).

**Metabolism**. Little information is available on the metabolism of gliclazide. At least eight metabolites (three major) have been identified by thin layer and gas-liquid chromatography. Some of these are glucuronide acid conjugates; only one of the metabolites has been identified (*p*-toluene sulfonamide). The liver is the probable site of metabolism.

**Excretion**. Approximately 70% of the administered dose appears to be excreted in the urine and 11% in the faeces. The urinary excretion of the drug is slow and the maximum rates do not occur until seven to ten hours after initial administration. The metabolic products are detectable in the urine 120 hours after oral administration. Faecal elimination is usually complete within 144 hours of oral administration.

**Indications** Diabetes mellitus of the maturity onset type, which cannot be controlled by diet alone.

**Contraindications** Gliclazide should not be used in diabetes complicated by acidosis, ketosis or coma, or in patients with a history of repeated episodes of ketoacidosis or coma.

**Juvenile onset diabetes and unstable or brittle diabetes**. As sulfonylurea hypoglycaemic agents are not effective in juvenile onset, unstable or brittle diabetes, gliclazide should not be used in these conditions.

**Severe renal and hepatic disease**. Gliclazide is contraindicated in severe hepatic or renal insufficiency. Caution should be exercised and dosage reduction may be required when using gliclazide in patients with impaired renal or hepatic function.

**Hypersensitivity**. Gliclazide should not be used with patients with known sensitivity to sulfonylureas.

**Use in pregnancy** (Category C) It is important to achieve strict normoglycaemia during pregnancy. Oral hypoglycaemic agents should be replaced by insulin.

The sulfonylureas may enter the fetal circulation and cause neonatal hypoglycaemia. In animal studies, embryotoxicity and/or birth defects have been demonstrated.

Gliclazide should not be used in pregnant women. Whilst animal studies of gliclazide have not shown any teratogenic effect, gliclazide should only be used in women who are likely to become pregnant if the benefits outweigh the potential risk.

**Use in lactation**. Gliclazide should not be used during lactation.

**Warnings Acute complications** such as severe trauma, fever, infection or surgery. These acute complications provoke additional metabolic stress which accentuate the predisposition to hypoglycaemia and ketosis. Patients presenting with such conditions may require insulin to maintain control. It is not appropriate to increase the dosage of gliclazide.

**Hypoglycaemia**. Close observation and careful initiation and adjustment of dosage is mandatory in patients who are elderly and debilitated, malnourished, semistarved or simply neglecting dietary restrictions. In such patients, severe hypoglycaemia may occur with all sulfonylureas and may require corrective therapy over a period of several days. Certain conditions such as alcoholism, insulinoma and adrenal, thyroid and pituitary insufficiency increase the sensitivity to sulfonylureas and may dispose to hypoglycaemia.

**Precautions Monitoring of diabetic state**. As with other antidiabetic therapies, patients must be under close medical supervision. Particular care must be taken during the initial period of stabilisation. Patients treated with gliclazide should be monitored regularly to ensure optimal control of the diabetic state, and where necessary, for adjustment of dosage.

**Transferring to gliclazide**. Patients who have been previously treated with sulfonylureas or biguanides alone or in combination may be transferred to gliclazide.

When gliclazide is administered as sole therapy to patients who have previously required combination therapy (e.g. biguanides and sulfonylureas), careful observation is essential during the transitional phase.

It is not generally recommended that insulin treated patients be transferred to gliclazide.

**Patient awareness**. Comprehensive instructions must be given to the patient about the nature of the disease and what must be done to detect and prevent complications.

**Interactions Disturbance of blood sugar control**. As with all hypoglycaemics, caution should be observed in administering thiazide diuretics to patients on gliclazide therapy, since thiazides have been reported to aggravate the diabetic state. Other drugs which may adversely affect blood sugar control with hypoglycaemic agents in some patients, include barbiturates, glucocorticoids and oestrogens. Potentiation of hypoglycaemic effect. Certain drugs may potentiate the effect of gliclazide and thereby increase the risk of hypoglycaemia. These include insulin, biguanides, sulfonamides, oxyphenbutazone, phenylbutazone, clofibrate, salicylates, coumarin derivatives, chloramphenicol, monocyclic oxidase inhibitors (MAOIs),  $\beta$ -blockers, cimetidine and ethanol.

**Alcohol**. Acute alcohol intoxication potentiates the hypoglycaemic action of all sulfonylurea agents. Furthermore, ingestion of alcohol may cause a disulfiram-like reaction with characteristic flushing of the face, throbbing headache, giddiness, tachypnoea, tachycardia or angina pectoris.

**Chronic alcohol abuse** may, as a result of liver enzyme induction, stimulate the metabolism of sulfonylurea drugs and shorten plasma half-life and duration of action.

**Adverse Reactions** Adverse reactions have occurred in some 12% of cases in clinical studies. However, approximately 2% of patients were withdrawn from therapy because of adverse reactions, notably hypoglycaemia, gastrointestinal disturbances (constipation, nausea, epigastric discomfort and heartburn), dermatological reactions (rash and transient itching), and biochemical abnormalities (elevated serum creatinine, increased serum alkalin phosphatase, raised serum AST, elevated BUN and raised serum bilirubin). Headache, slight disulfiram-like reactions and lassitude have also been reported.

Serious reactions which have been reported with other sulfonylureas are leucopenia, thrombocytopenia, agranulocytosis, pancytopenia, haemolytic anaemia, cholestatic jaundice and gastrointestinal haemorrhage. These reactions have not been reported with gliclazide.

As is the case with all forms of antidiabetic therapy, hypoglycaemic reactions have occasionally been reported following Diamicron administration.

Severe hypoglycaemia, though very rarely reported, may occur in patients receiving gliclazide.

**Dosage and Administration** The dosage of gliclazide should be carefully titrated to maintain optimal control at the various possible dose levels. Dosage should be initiated at 40 mg (half tablet) daily and may be increased if necessary up to 320 mg (four tablets) daily. Doses up to 160 mg daily may be taken in a single dose but preferably at the same time each morning. Doses in excess of 160 mg should be taken in divided doses in the morning and evening.

In general, the dosage will depend on the severity of the glycaemia with ongoing adjustments made in order to obtain the optimal response at the lowest dosage.

Treatment with gliclazide does not obviate the necessity for maintaining standard dietary regulations.

**Overdosage Symptoms**. Manifestations of severe hypoglycaemia result from overdosage. Hypoglycaemia caused by sulfonylurea agents differs in several aspects from insulin coma. Warning symptoms are often absent, neurological syndromes are frequent and coma is often prolonged.

**Treatment**. Consciousness should be restored by the administration of intravenous glucose or glucagon injection, care being taken to ensure against the return of hypoglycaemia by constant monitoring of the blood sugar level.

**Presentation** Tablets, 80 mg (white, scored): 100's.

**Poisons Schedule S4.**

TGA approval/last amendment: 21/08/2001

### Diamicron MR

Servier Laboratories (Aust.) Pty Ltd

**Composition** Active. Gliclazide.

Inactive. Calcium hydrogen phosphate, maltodextrin, hydrogenated magnesium stearate, colloidal anhydrous silica.

**Description** Chemical name: 1-(3-azabicyclo[3.3.0]oct-3-yl)-3-p-tolylsulfonylurea. Molecular formula:  $C_{15}H_{21}N_3O_3S$ . Melting point: approximately 168°C. CAS: 21187-98-4. Gliclazide is a white almost white powder, practically insoluble in water, freely soluble in dichloromethane, sparingly soluble in acetone and slightly soluble in ethanol 96%.

**Actions Pharmacology**. Gliclazide is an oral hypoglycaemic agent which differs from other related compounds in that it contains a heterocyclic ring with an endocyclic bond.

Gliclazide reduces blood glucose levels by stimulating insulin secretion from the  $\beta$ -cells of the islets of Langerhans.

Gliclazide shows high affinity, strong selectivity and reversing binding to the  $\beta$ -cell K<sub>ATP</sub> channels with a low affinity for the vascular K<sub>ATP</sub> channels. Increased postprandial insulin and glucose secretion persists after two years of treatment. Gliclazide also has extrapancreatic effects and haemovascular properties.

**Effects on insulin release**. In type II diabetes, gliclazide restores the first peak of insulin secretion in response to glucose and increases the second phase of insulin secretion. A significant increase in basal insulin release is seen in response to stimulation induced by a meal containing glucose.

**Extrapancreatic effects**. Gliclazide has been shown to improve peripheral insulin sensitivity.

In muscle, euglycaemic hyperinsulinaemic clamp studies with gliclazide have demonstrated significantly increased glucose uptake which may improve glucose disposal. Gliclazide potentiates insulin action on muscle glycogen synthase. These effects are consistent with a post-transcriptional effect of gliclazide on GLUT4 glucose transporters.

Studies on glucose turnover have further shown that gliclazide decreases hepatic glucose production, leading to an improvement in fasting blood glucose levels.

**Other actions**. Gliclazide has been shown in some studies to have actions independent of that on glucose levels. These haemovascular effects of gliclazide include the following.

Partial inhibition of platelet aggregation and adhesion with a reduction in markers of platelet activation ( $\beta$  thromboglobulin, thrombinogen). Increased vascular endothelial fibrinolytic activity (increased t-PA).

Antioxidant properties, notably a reduction in plasma lipid peroxidation and increased erythrocyte superoxide dismutase activity. Inhibition of the increased adhesiveness of type II diabetes patients' monocytes to endothelial cells *in vitro*.

The antioxidant, platelet inhibiting and fibrinolytic actions of gliclazide involve processes which have been implicated in the pathogenesis of vascular complications of type II diabetes.

There is no clinical evidence that the haemovascular effects of gliclazide are of therapeutic benefit in type II diabetes patients.

**Pharmacokinetics**. Hydration of the tablets induces formation of gel to activate drug release.

Plasma levels increase progressively, resulting in a plateau in the curve from the sixth to the twelfth hour after administration. Individual variability is low. Gliclazide is completely absorbed. The oral bioavailability does not affect the rate or degree of absorption. The relationship between the dose administered and the area under the concentration (AUC) curve as a function of time is linear for gliclazide up to 90 mg/day. At the highest evaluated dose (160 mg/day), the AUC increases slightly more than proportionally.

Plasma protein binding is approximately 95%. Gliclazide is extensively metabolised in the liver, the products of which are excreted in the urine. Less than 1% of unchanged drug is recovered in the urine. No active metabolites have been detected in plasma. The clearance of gliclazide has been found to be slightly reduced as a function of age. This reduction, however, is not considered clinically significant.

The elimination half-life of gliclazide is approximately 16 hours. No clinically significant modifications in the pharmacokinetic parameters have been observed in elderly patients.

**Indications** Type II diabetes in association with dietary measures when dietary measures alone are inadequate to control blood glucose levels. During controlled clinical trials in patients with type II diabetes, Diamicron MR, taken as a single daily dose, was shown to be effective long term in controlling blood glucose levels based on monitoring of HbA1c.

**Contraindications** Hypersensitivity to gliclazide, other sulfonylureas, sulfonamides or to any of the excipients.

Type I diabetes, diabetic ketoacidosis, diabetic pre-coma, severe renal or hepatic insufficiency.

Treatment with miconazole (see Interactions).

Pregnancy and lactation (see Use in pregnancy and Use in lactation). It is generally not recommended to use this agent in combination with phenylbutazone or danazol (see Interactions).

**Use in pregnancy** (Category C) It is important to achieve strict normoglycaemia during pregnancy. Oral hypoglycaemic agents should be replaced by insulin. The sulfonylureas may enter the fetal circulation and cause neonatal hypoglycaemia. In animal studies, embryotoxicity and/or birth defects have been demonstrated with some sulfonylureas.

Gliclazide should not be used in pregnant women although animal studies of gliclazide have not shown any teratogenic effect. From a clinical point of view, there are no adequate data to allow evaluation of the possible malformative or fetotoxic effects of gliclazide when administered during pregnancy.

**Use in lactation**. In the absence of data on the transfer of gliclazide into breast milk, and given the risk of neonatal hypoglycaemia, breastfeeding is contraindicated during treatment with this product.

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